Three common phenotypes - HP 1-1, HP 2-1, and HP 2-2 are found for haptoglobin. Two co-dominate alleles Hp^1 and Hp^2 control these phenotypes. Are shown in Figure 8, all 50 dolphin samples examined exhibited the HP 1-1 phenotype, suggesting that the Hp^2 allele is not present in this population.

I. Plasma Protein Electrophoretic Profiles

As illustrated in Figure 9,5 different electrophoretic profiles were obtained from the dolphins in this study. Column A represents the profile "expected" under the conditions for these analyses. Of the 49 samples analyzed, 13 (27%) exhibited this profile (one sample, number 603 was too badly hemolyzed to get meaningful results and was thus not included in the figure). The profile most frequently encountered contained on "extra" band between the α_1 and α_2 zones. Thirty two of the samples (65%) exhibited this pattern. An extra band between the α_2 and β zones was found in two samples (4%). One sample (2%) contained an extra band between the β and α_2 as well as between the α_1 and α_2, and another sample (2%) contained extra bands between the β and γ, and between the α_1 and α_2 zones.

As discussed previously, agarose electrophoresis followed by a general protein stain should only be considered as a screening technique for identifying those samples to be selected for additional characterization, including possible genetic variability. Conclusions based solely on this technique are difficult to make for 2 reasons. First, each of the bands observed in this technique is a mixture of many different proteins exhibiting similar electrophoretic mobilities. Thus, observed

mobility differences can not be directly correlated to only 1 protein or enzyme as can be done with the other techniques used in this study. Second, mobility differences and extra bands are caused not only by genetic variabilities, but are also indicative of various disease states and even recent diets of the individual. In addition, a variety and/or combination of disease states and genetic variability can produce similar agarose electrophoretic patterns. Thus, one cannot assume that all dolphins exhibiting similar patterns exhibit the same genetic makeup or similar clinical symptoms.

While exact conclusions can not be drawn regarding any of the specific patterns from this study, some general conclusions and recommendations for additional studies can be made from these experiments. The "extra" band formed between α_2 and α_1 which was apparent in 32 of the 50 samples was in the region where one would expect trypsin and chymotrypsin inhibitors, ceruloplasmin and/or the C_1 component of complement. From a genetic variability standpoint, the most interesting of these possibilities are the protein inhibitors, particular α-1-antitrypsin inhibitor(AAT). A number of phenotype patterns of AAT exist in the human population. Each of these patterns can be explained in terms of a single locus with multiple alleles which are co-dominately expressed. Since the function of this protein is to inhibit proteolytic enzymes in serum, and since different phenotypes exhibit varying degrees of inhibitor capacity, some phenotypes predispose towards lung and liver disease. Thus, if this band is indeed AAT, not only would future studies which include AAT phenotyping aid in the genetic characterization of dolphins, but would also allow for prediction as to possible predisposition towards lung and liver diseases.

The "extra" band observed between the α_2 and β zones in four dolphins (604, 616, 647, 650) is most probably transferrin or a transferrin variant. The function of this protein is to transport iron, mostly from the intestine to the bone marrow where it is utilized in the synthesis of hemoglobin and myoglobin. A number of transferrin phenotypes exist in humans as well as other vertabrates. The preliminary data from this study suggests that such transferrin variability might also exist in dolphins, and the inclusion of transferrin phenotyping in future studies is justified.

Finally, the "extra" band observed between the β and γ zones of samples 647 and 650 was probably due to either fibringen or the C_3 component of complement. Two common variants and a number of less common variants of fibringen have been detected. While future studies to identify this band in 647 and 650 are certainly justified, wide scale screening of C_3 and fibringen phenotypes do not appear to be warranted at this time.

Genetic Applications

Table 1 lists the RBC and plasma proteins examined, and the number of loci involved. In addition, the variability of these proteins is indicated. As can be noted from the table, approximately 50% of the loci examined were variable. In the 1979 tagging program conducted by Sea World and Hubbs/Sea World Research Institute, approximately 30% variability was noted. The increased variability in this study is due to our finding of 2 variable proteins which were scored monomorphic in the previous studies. In our study, 1 sample of Glucose-6-phosphate dehydrogenase was found to have a definite mobility difference and was

thus scored as a variable loci. In the previous study, this enzyme was scored as monomorphic, even though it was noted that a possible mobility difference might exist.

The most significant difference between this study and the 1979 study is our finding of variable Hemoglobin Loci. Most probably, this is due to the selection of isoelectric focusing in this study in place of starch gel electrophoresis used in the 1979 study. Quite probably, this technique revealed small difference in protein structure which were not readily apparent with the techniques utilized in 1979. However, it should be emphasized that this Loci variability might not exist in Tursiops in the Indian River vicininty, and/or is limited to Tursiops in the northern Gulf of Mexico. Obviously, future studies should employ appropriate techniques for hemoglobin phenotyping which will answer these questions.

Table II summarizes the allele frequencies for those isoenzymes which exhibited Loci variability. Of the four proteins scored, only allele frequencies for Esterase were reported for the 1979 study [GLO and MDH were not resolved sufficiently by these workers for scoring and no variability found in hemoglobin]. However, the similarity on Esterase D in this study and that reported for the 1978-1979 studies are remarkably smiliar. The 1978-1979 study reported an allele frequency for ESD *1 (m^1) of 0.73 and ESD *2 (m^3) of 0.27. These frequencies in this study were found to be 0.77 and 0.73 respectively. This observation is especially interesting since the 1979 study found patterns of local differentiation. In light of the present study, it would appear that such differences were probably due to sampling and/or the small

number of animals tested [8 and 26], rather than the suggested local variation.

Unfortunately, only 2 presumptive calves were collected in this study, and thus little can be concluded about interrelationships within capture units in terms of predicted Mendelian inheritance requirements for alleles involved.

648-650	648 = calf. This calf is heterozygous for glyoxylase I. Since both 649 (presumptive mother) and 650 (possible mother) are homozygous for this enzyme [GLO 2], the father must contain a GLO 1 allele. The only male exhibiting this allele collected in this study was 633.
626-628	626 = calf. Both calf and mother (628) were homozygous for all alleles tested. Little can be concluded about father.
603-605	604 sample hemolyzed on arrival, no conclusive analyses could be run. Number 604 could be calf of 605 since data fit with mother-son relationship.
608-609	This unit both males. 609 is only animal to exhibit hemoglobin with α chain abnormality
636-640	Animals 636 and 640 were the only two animals collected which exhibited a substitution on the β chain of Hb. This suggests that 640 might be the mother of 636. Enzyme data do not eliminate this possibility.

TABLE I

SUMMARY OF ENZYME/PROTEINS EXAMINED

	Number Loci
A. Monomorphic loci (no variants found)	
1. Haptoglobin	1
2. 6-Phosphogluconate Dehydrogenase	1
3. Lactate Dehydrogenase	2-3
4. Albumin	1
5. Post Albumin	1
B. Variable Loci	
1. Hemoglobin	2-3
2. Glyoxylase	1
3. Esterase D	1
4. Glucose-6-phosphate Dehydrogenase (mobility difference on 1 sample)	1
5. Malate Dehydrogenase	1

TABLE II

SUMMARY OF PHENOTYPES AND ALLELE FREQUENCIES

Enzyme	Phenotype	Allele Frequency *(combined)
Esterase D (3.1.1.1)	ESD 1 62% ESD 2-1 30% ESD 2-2 8%	ESD *1 0.77 ESD *2 0.23
Glyoxylase - 1 (4.4.1.5)	GLO 2 90% GLO 2-1 10% GLO 2-2 0%	GLO *1 0.95 GLO *2 0.05
Malate Dehydrogenase (NAD) (Soluble) (1.1.1.37)	MDH I 1 92% MDH 1 I-3 2% MDH 1 3 6%	MDH 1 *I 0.93 MDH 1 *3 0.07
Hemoglobin	HBA 94% HBA/HBA* 4% HBB/HBB* 2%	HBA 0.98 HBA* .02 HBB .99 HBB* 0.01

Figure 1

HEMOGLOBIN SEPARATION BY ISOELECTRIC FOCUSING

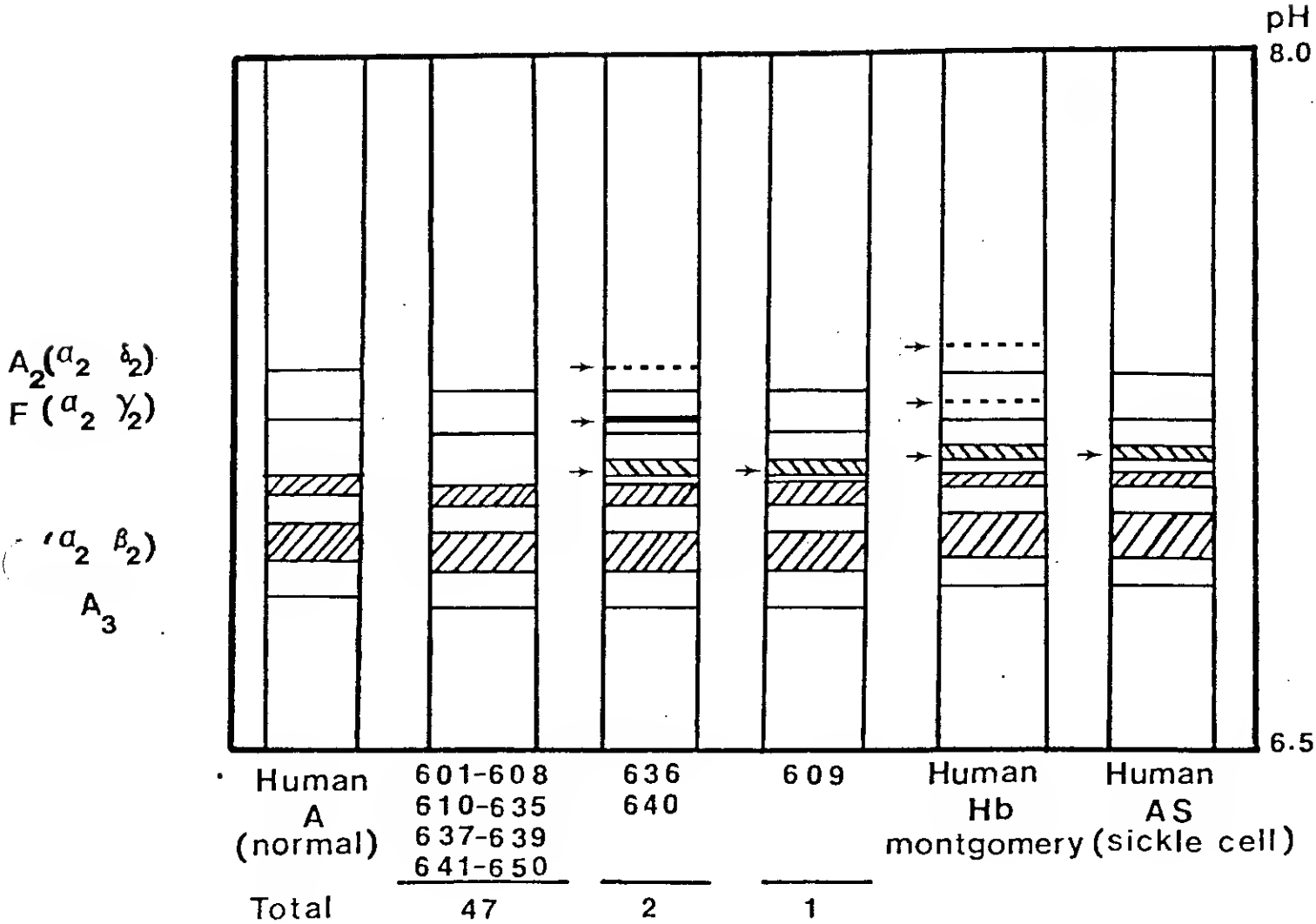


Figure 2

LACTATE DEHYDROGENASE ISOENZYME PROFILES

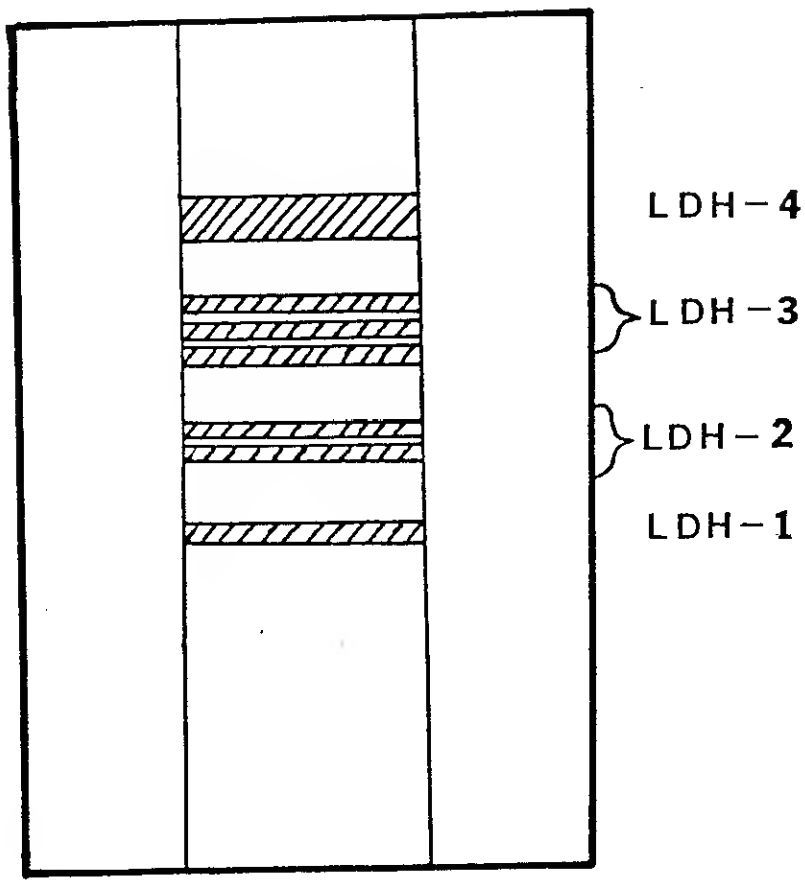
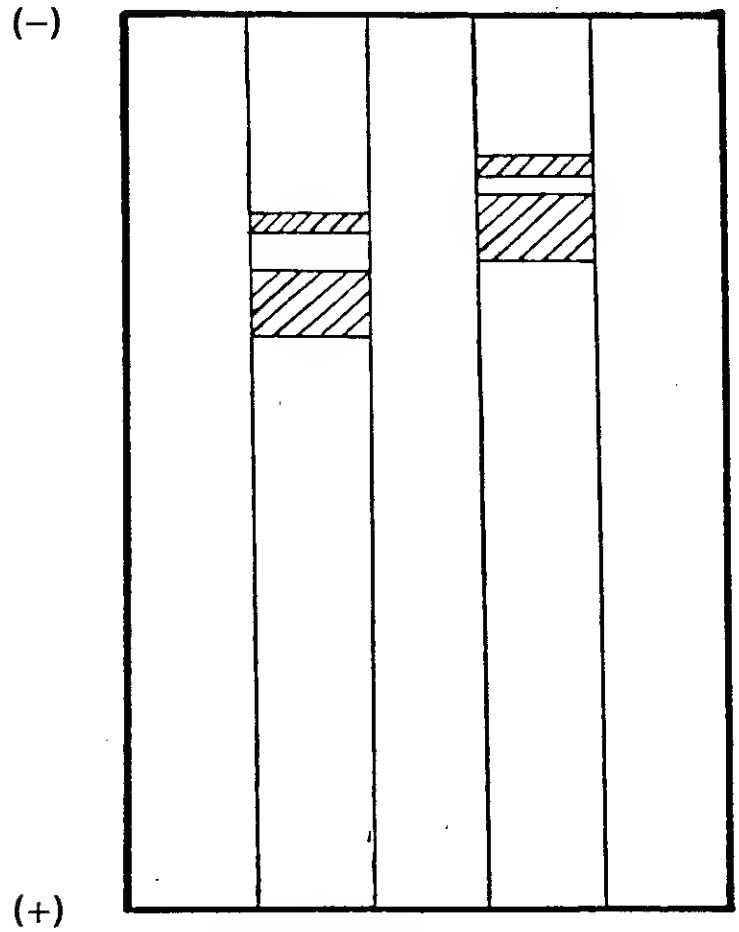


Figure 3

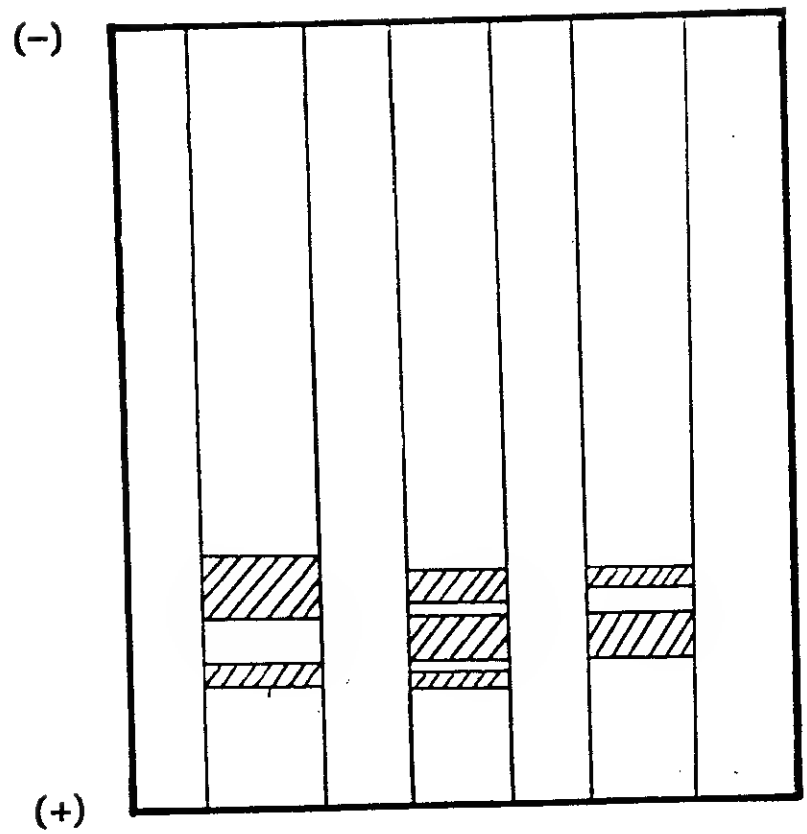
GLUCOSE-6-PHOSPHATE DEHYDROGENASE PHENOTYPES



	601–635 637–650	636
Total	49	1

Figure 4

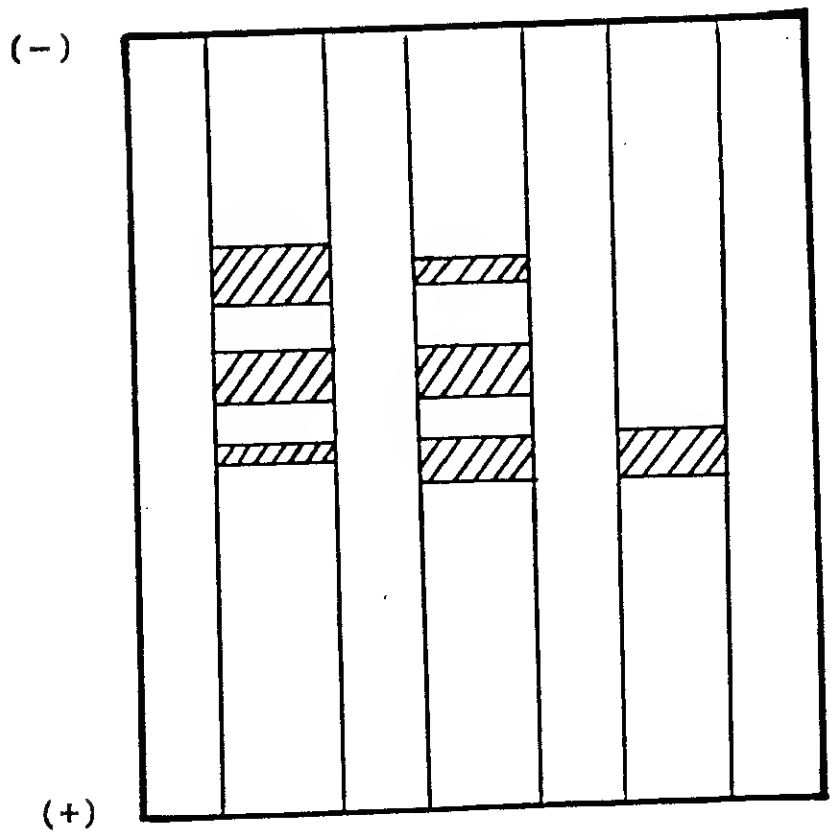
ESTERASE D PHENOTYPES



Type	ESD-1	ESD-2-1	ESD-2
Samples exhibiting Pattern	601 602 604-620 626-630 631-636 640	604 621-625 641-643 645-650	637-639 644
Total	31	15	4

Figure 5

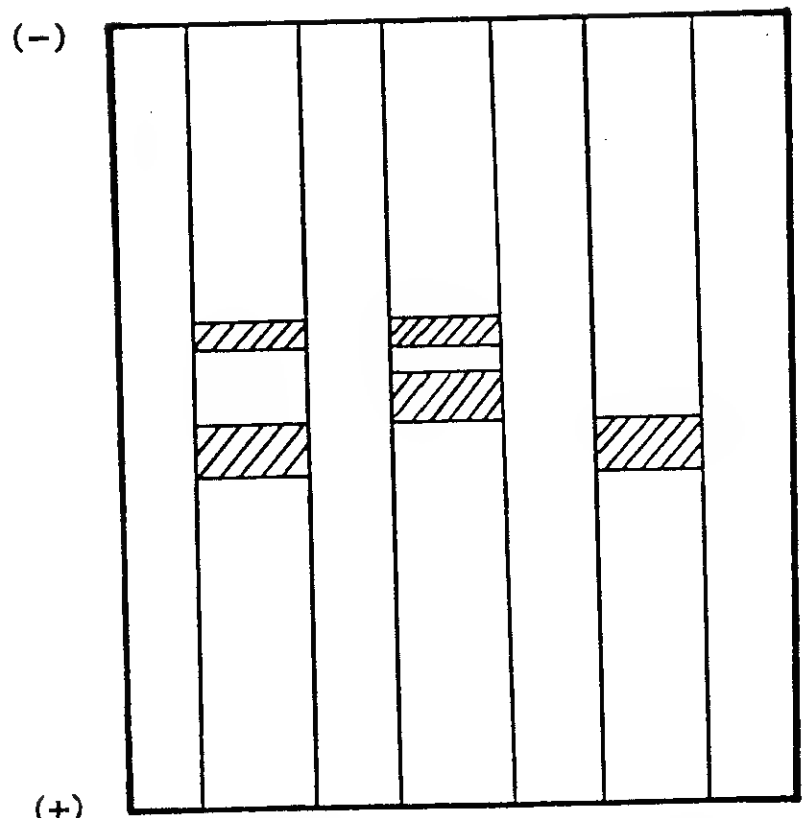
6-PHOSPHOGLUCONATE DEHYDROGENASE PHENOTYPES



Type	C	AC	A
Samples exhibiting pattern	none	none	601-650
Total	0	0	50

Figure 6

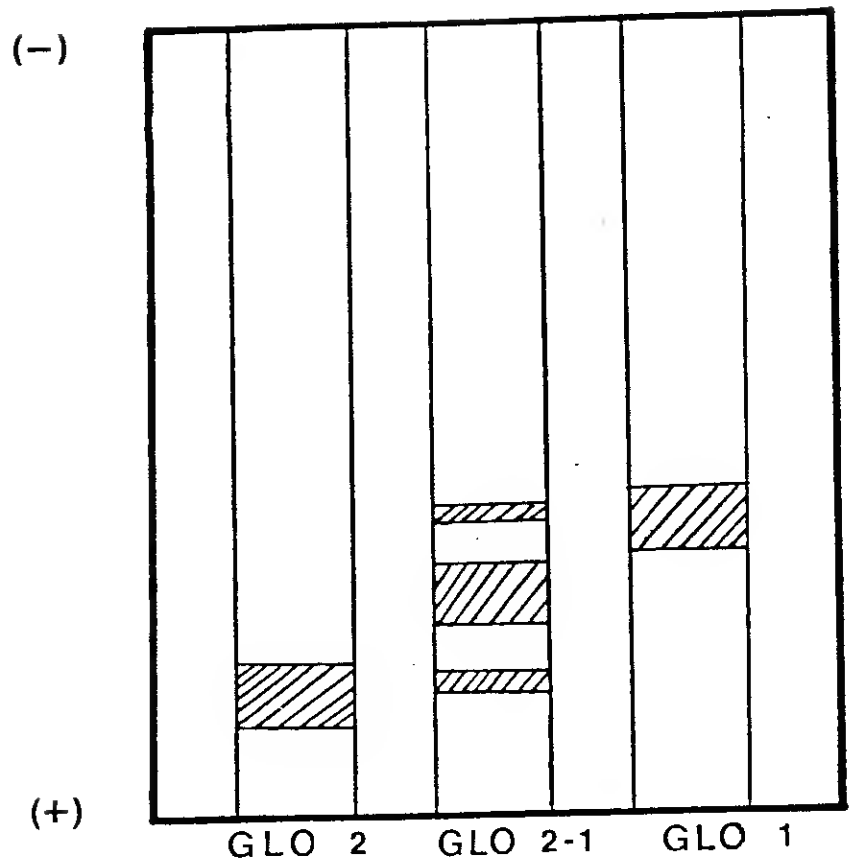
MALATE DEHYDROGENASE PHENOTYPES



Total	46	1	3

Figure 7

GLYOXALASE I PHENOTYPES



	GLO 2	GLO 2-1	GLO 1
	601 - 631	632	
	634 - 636	633	
	638 - 643	637	
	645 - 647	644	
	649 - 650	648	
Total	45	5	0

Figure 8

HAPTOGLOBIN PHENOTYPES

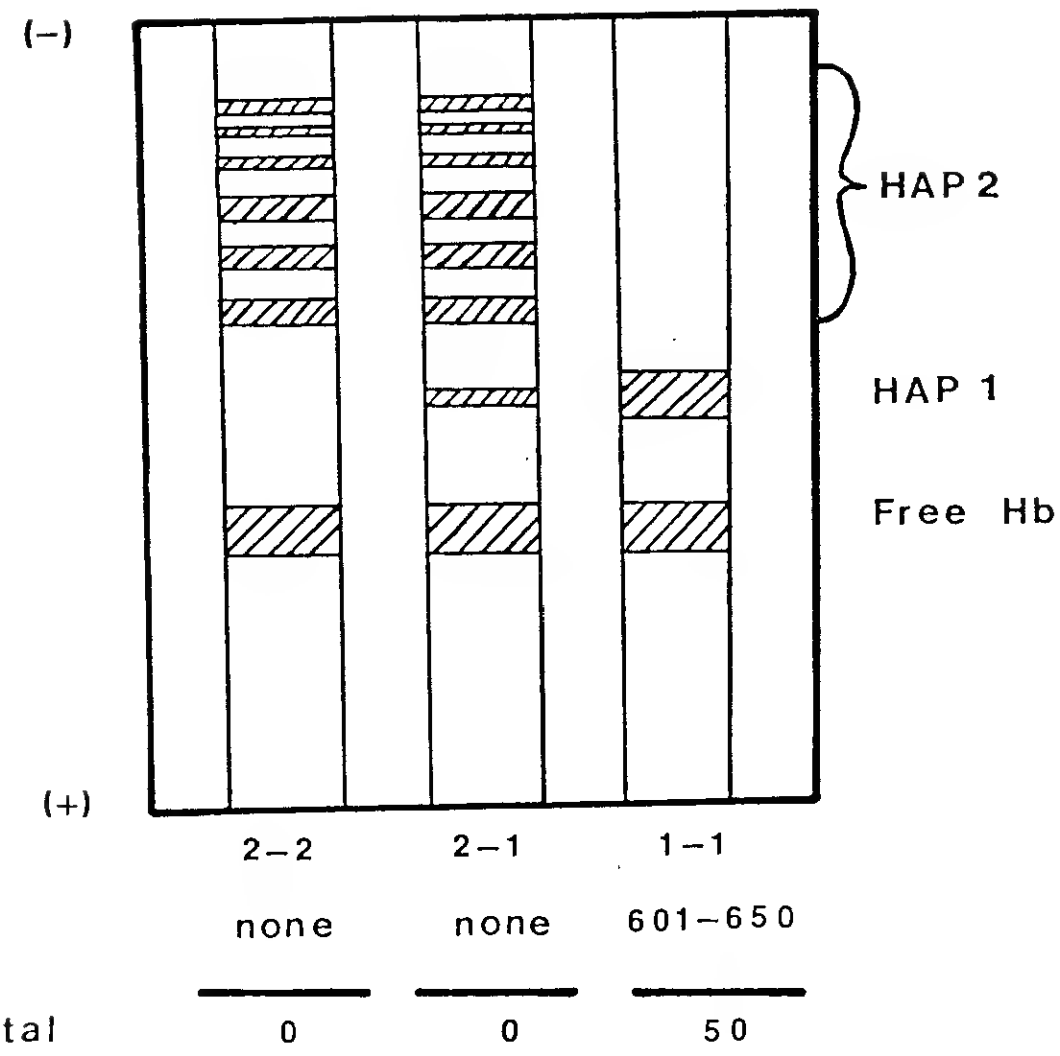
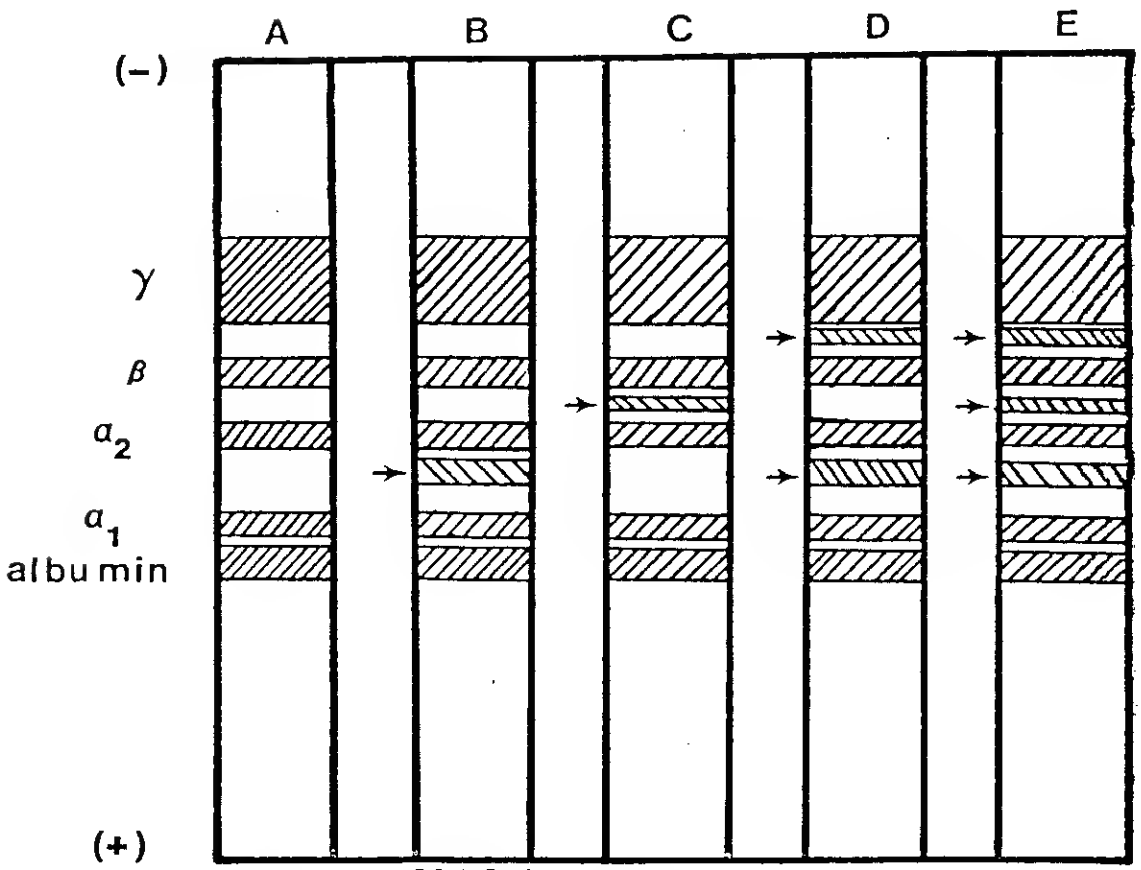


Figure 9

PLASMA PROTEIN ELECTROPHORESIS PROFILES



	A	B	C	D	E
	601	605-608	604	647	650
	602	610-614	616		
	609	617-620			
	615	624-628			
	621-623	632-640			
	629-631	644-646			
	641-643	648-649			
Total	13	32	2	1	1

AGE ESTIMATION AND HORMONE ANALYSES FOR BOTTLENOSE DOLPHINS, *Tursiops truncatus* FROM MISSISSIPPI

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OBJECTIVES

The objectives of this study were: 1) to estimate the ages of bottlenose dolphins captured, marked, and released in the Mississippi Sound, and 2) to estimate the blood serum concentrations of testosterone, estrogen (estradial), and progesterone as appropriate for the sex of the animal.

METHODS

Age Estimation

One maxillary tooth was extracted from the lower jaw of the animal. If a tooth broke during extraction (for example no. 617) it was considered taken. Also, no teeth were taken from dolphins no. 651 to 653. Approximately 10 minutes prior to extraction, the tooth was blocked by injecting a 2% solution of Xylocaine (Lidocaine) at the base of the tooth. During the anesthetizing and extracting processes the mouth was kept open by placing between the jaws a 2 inch diameter wooden gag covered with a 2¼ inch diameter soft-plastic hose. After the tooth was pulled out, the excavated area of the gum was packed with resorbable material containing antibiotics.

To serve as a future age-marker and precautionary antibiotic, each animal was injected with a dose of oxytetracycline at the rate of 15 mg. per kg body weight. Because of the volume of the antibiotic used, more than one site in the musculature was used to administer the drug.

The extracted teeth were boiled in tap water for about 5 minutes and then the adhering tissue was scraped off with a dull instrument. The teeth were stored in a 50:50 mixture of 70% ethanol and glycerol to which a few crystals of thymol had been added to retard fungal growth. The fluid served to prevent the teeth from drying out and cracking.

Cleaned teeth were mounted on a chuck for a Buehler Isomet Low Speed saw (Buehler, Ltd., Lake Park, Illinois) with Buehler Thermoplastic cement. Each tooth was sectioned longitudinally with the Buehler high density diamond blade. At least one thin section (approximately 150 um) was cut from each tooth. We tried to take the thin sections from the center of each tooth. Thin sections and half teeth were returned to the ethanol-glycerol mixture for storage.

Growth layer groups were counted in the thin sections using a Wild binocular dissecting microscope equipped with polarizing filters and using transmitted light. The storage solution served as the temporary mounting medium. After examination, the sections were returned to the storage vials. In a few cases additional thin sections were cut to verify growth layer group counts. All thin sections were examined without reference to the length or sex of the animal.

After the thin section examination was completed, a half-tooth from each animal was etched for 3 hours in 5% formic acid. Care was taken to thoroughly rinse the storage fluid from the tooth

before placing it in the formic acid bath. After etching, the teeth were rinsed in tap water for at least one hour and then air dried overnight to bring out the surface relief. The teeth were examined under the Wild M-5 with oblique lighting. Growth layer group counts obtained from the etched teeth were compared with those from the thin sections. Sections were reexamined as necessary until the best count was obtained. Etched teeth were returned to the storage solution.

Endocrinology

Serum samples were received via overnight carrier in styrofoam containers. The samples arrived frozen on dry ice and were immediately stored in a Revco freezer (-170° C) until assayed. Testosterone and progesterone were assayed directly from serum by radioimmunoassay (RIA), while estradiol was first extracted with a hexane/ethyl acetate solvent before being assayed by RIA. Kits for each RIA were obtained from Radioassay Systems Laboratories, Inc. (Carson, Ca) and used I-125 as the tracer label. Standard curves for each hormone assayed are shows in Figures 7-9 and were used to interpolate the values for the unknown samples. The data was processed using a computer program obtained from the National Institutes of Health and modified to run on our Univac computer. Protocols for each assay are included in the Appendix.

RESULTS AND DISCUSSION

Age Estimates

The age estimates obtained from both thin sections and etched teeth are given in Table 1 along with the animal number, length, sex, weight and hormone concentrations. In general, the growth layer counts obtained from the thin sections were in close agreement with those obtained from the etched teeth. In a few cases (e.g. animal 645) the estimates differed greatly and the highest count was used. Generally the etched tooth gives a higher count in the older animals in which the pulp cavities are often occluded. In Table 1, a '+' symbol following an age estimate indicates that the pulp cavity was occluded and growth layers could not be counted beyond the number given. A '>' symbol preceeding an age estimate indicates that the animal was probably early into the next year's growth (e.g. >5 means that the animal is just starting its 6th year). The length-age data are plotted in Figues 1-3 for all animals (Fig. 1) males, (Fig. 2), and females (Fig. 3). The results are somewhat biased because animals aged as n+ years were plotted as being 'n' years old. The same bias applies to the endocrine data in Figures 4, 5, and 6. The overall length-age data is similar to that obtained by Odell and Asper (1982) in that most of the growth in length occurs during the first 2 years of life. We get the impression that adult females from Mississippi may be shorter than adults in the Indian River (e.g. animal 612 is 233 cm and 22 years old).

However, the sample of old adult females is small. Data from stranded animals could be used to test this possible difference.

ENDOCRINOLOGY

Results of the radioimmunoassays for testosterone in males, and estradiol and progesterone in females are listed in Table 1. Serum hormone concentrations are plotted against age in Figures 4-6.

Due to the small sample size, conclusions regarding the observed hormone levels are difficult to make and extremely tenuous. For example, only three males had testosterone levels in the range for normal human adults (3-10 ng/ml) and all were greater than nine years of age. This is obviously not a statistically significant sample. Between 5 and 9 years, males tended to show typical prepubertal levels (0.1-0.2 ng/ml) and in younger animals, testosterone was not measureable. More samples are needed to clearly define the age of maturation in males.

Serum estradiol in females tended to increase with age with higher levels starting at approximately 5-6 years of age. Similarly, serum progesterone increases after age 6, and together these data indicate the onset of puberty in this population.

However, both estrogen and progesterone fluctuate widely during a reproductive cycle, which precludes any firm conclusions from single samples without larger samples sizes and some knowledge of the normal reproductive cycle in dolphins. Furthermore, the estradiol levels reported in this study are significantly lower

than those found in Indian River populations by Odell and Asper (1982). However, the details of the assays used in that study are notavailable, making direct comparisons unjustified at this time. In addition, there may be an error in the data report for 1980 Indian River estradial assays that would account for most of the apparent differences.

Data from this study and from that of Odell and Asper (1982) would be much more meaningful if normal endocrine fluctuations in dolphin reproductive cycles were known; perhaps from detailed and controlled studies on captive animals. Since the details of the dolphin estrous cycle, or indeed if it even exists, are not currently known, it is impossible to predict the reproductive status of an individual from a single sample, with the exception of differentiating prepubertal from adult animals. We thus suggest that a reproductive cycle profile for steroid and pituitary hormones be established in conjunction with a continued sampling program.

Literature Cited

Odell, D. K. and E. D. Asper. 1982. Live capture, marking and resighting of bottlenose dolphins, Tursiops truncatus. Final Report. National Marine Fisheries Service Contract NA80-GA-C-00063, 325 pp.

TABLE 1. SUMMARY OF AGE ESTIMATES AND HORMONE ANALYSES FOR BOTTLENOSE DOLPHINS FROM MISSISSIPPI.

				AGE (YRS.)		HORMONES		
ANIMAL	SEX	LENGTH (cm)	WEIGHT (kg)	T-SECTION	ETCH	TEST. (ng/ml)	EST. (pg/ml)	PROG. (ng/ml)
601	F	247	140	10	16		10.0	0.07
602	F	221	136	9	-		9.7	0.14
603	M	208	104	3	3	ND		
604	M	208	100	3	3	ND		
605	F	244	149	9	8		7.9	0.41
606	F	212	104	5	5		5.2	ND
607	M	207	111	5	5	0.16		
608	M	247	159	9	9	0.20		
609	M	214	131	8.5	8	0.20		
610	F	246	168	8+	12		27.7	ND
611	F	237	152	8+	8+		23.9	0.56
612	M	233	170	10+	22	lost sample		
613	M	255	222	7	12	12.54		
614	F	217	136	7	7		18.8	21.46
615	M	212	120	6	5	lost sample		
616	F	216	111	6	6		10.7	18.23
617	F	249	140	X			14.1	41.58
618	F	232	161	11+	11+(14?)		16.5	25.18
619	M	221	122	4	4	0.12		
620	F	220	118	4	4		ND	ND
621	M	214	127	4	4	0.08		
622	M	218	136	6	6	0.10		
623	F	222	129	X			26.7	0.17
624	M	239	152	7	7	0.28		
625	F	208	102	3	3		8.3	0.07

REMARKS:

TEST -E[illegible]DIOL; PROG.-PROGES[illegible]ERONE; X-NOT DETERMINED

TABLE 1. (CONTINUED)

ANIMAL	SEX	LENGTH (cm)	WEIGHT (kg)	AGE (YRS.) T-SECTION	AGE (YRS.) ETCH	HORMONES TEST. (ng/ml)	HORMONES EST. (pg/ml)	HORMONES PROG. (ng/ml)
626	F	193	91	2	2		16.8	0.14
627	F	222	152	10+	9		23.6	ND
628	F	230	140	14	15		29.7	16.48
629	F	216	116	-	6		23.3	ND
630	M	232	143	4	4	ND		
631	M	215	120	4.5	4	ND		
632	F	229	136	7	7		21.0	ND
633	M	246	204	15+	16+	7.8		
634	F	224	165	11	10-11		22.8	19.96
635	M	257	220	X	X	6.28		
636	M	216	116	7	7	0.08		
637	F	248	222	11+	8-10+		19.2	0.12
638	F	254	193	-poor	6+		12.3	2.33
639	F	248	204	13+ poor	8+		16.6	1.09
640	F	236	174	15+	15+		13.8	0.07
641	F	235	132	10	10		15.0	24.59
642	M	222	131	7-8	7	0.14		
643	M	221	136	8	8	0.10		
644	F	232	152	15+	15+		18.0	8.62
645	F	239	177	8	21		18.7	14.79
646	F	230	145	8	8		6.6	0.17
647	F	215	116	5	5		18.5	ND
648	F	232	170	5? poor	6?		14.6	15.49
649	F	208	116	3	3		10.1	0.08
650	F	216	129	5?	5		17.9	0.15
651*	F	226	122	X	X		10.0	12.10
652*	M	241	132	X	X	32.0		
653*	F	X	159	X	X		75.0	30.65

*Tests conducted by Pathology Laboratory, Hattiesburg, MS and are not part of data analysis
ND - Not Detectable
- - Not Determined

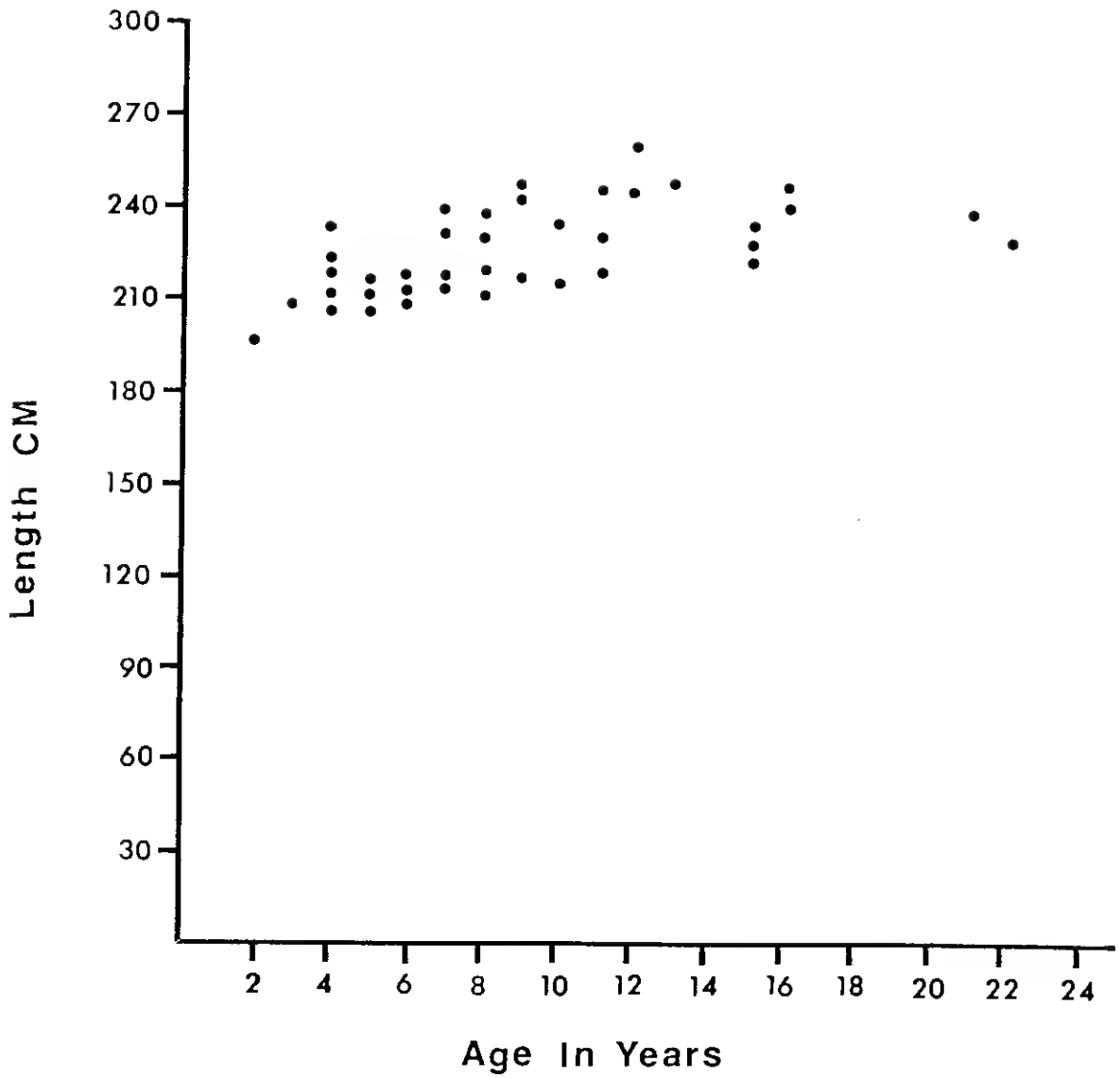


FIGURE 1: LENGTH-AGE RELATIONSHIP FOR BOTTLENOSE DOLPHINS CAPTURED IN MISSISSIPPI SOUND.

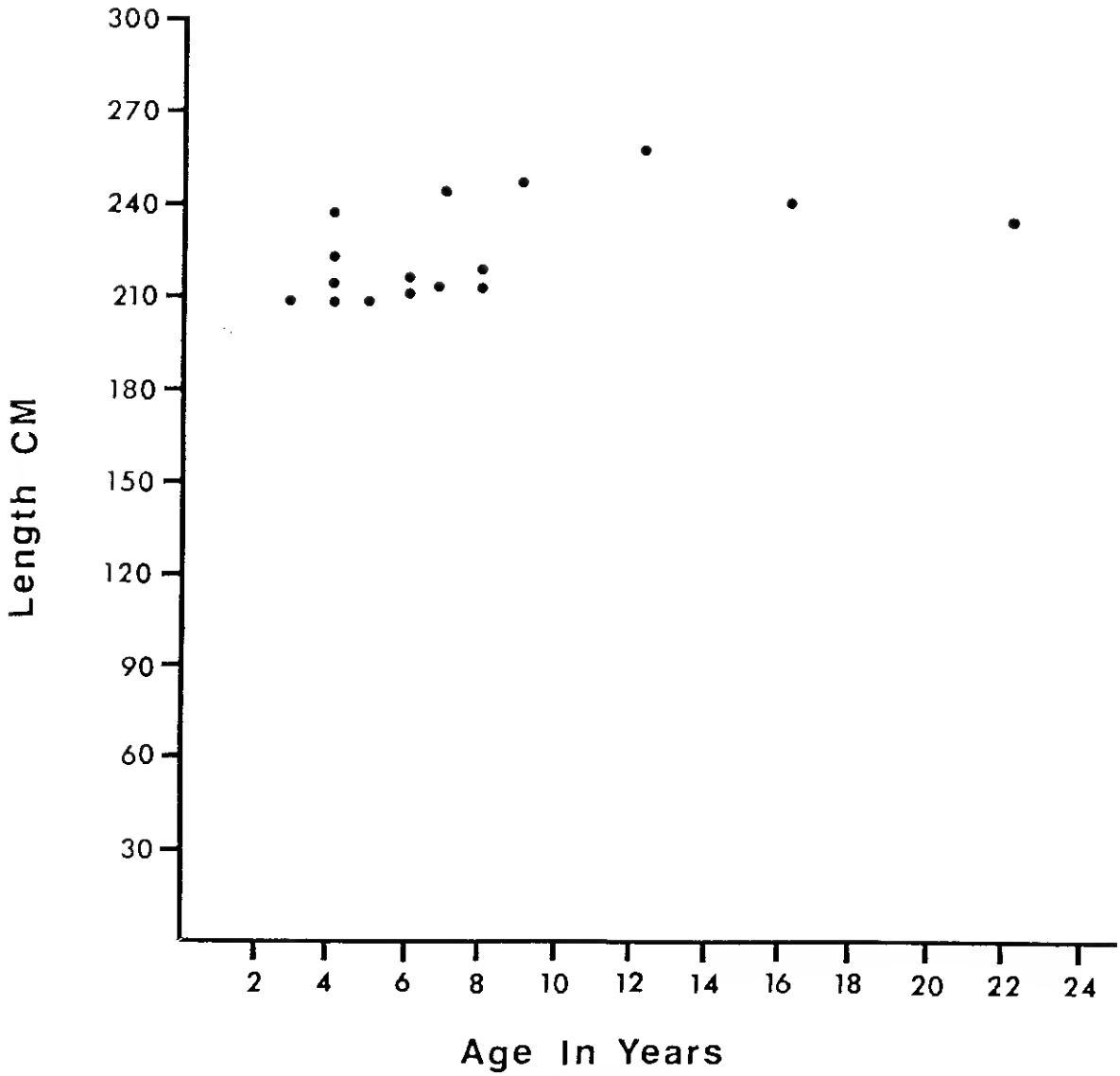


FIGURE 2: LENGTH-AGE RELATIONSHIP FOR MALE BOTTLENOSE DOLPHINS CAPTURED IN MISSISSIPPI SOUND.

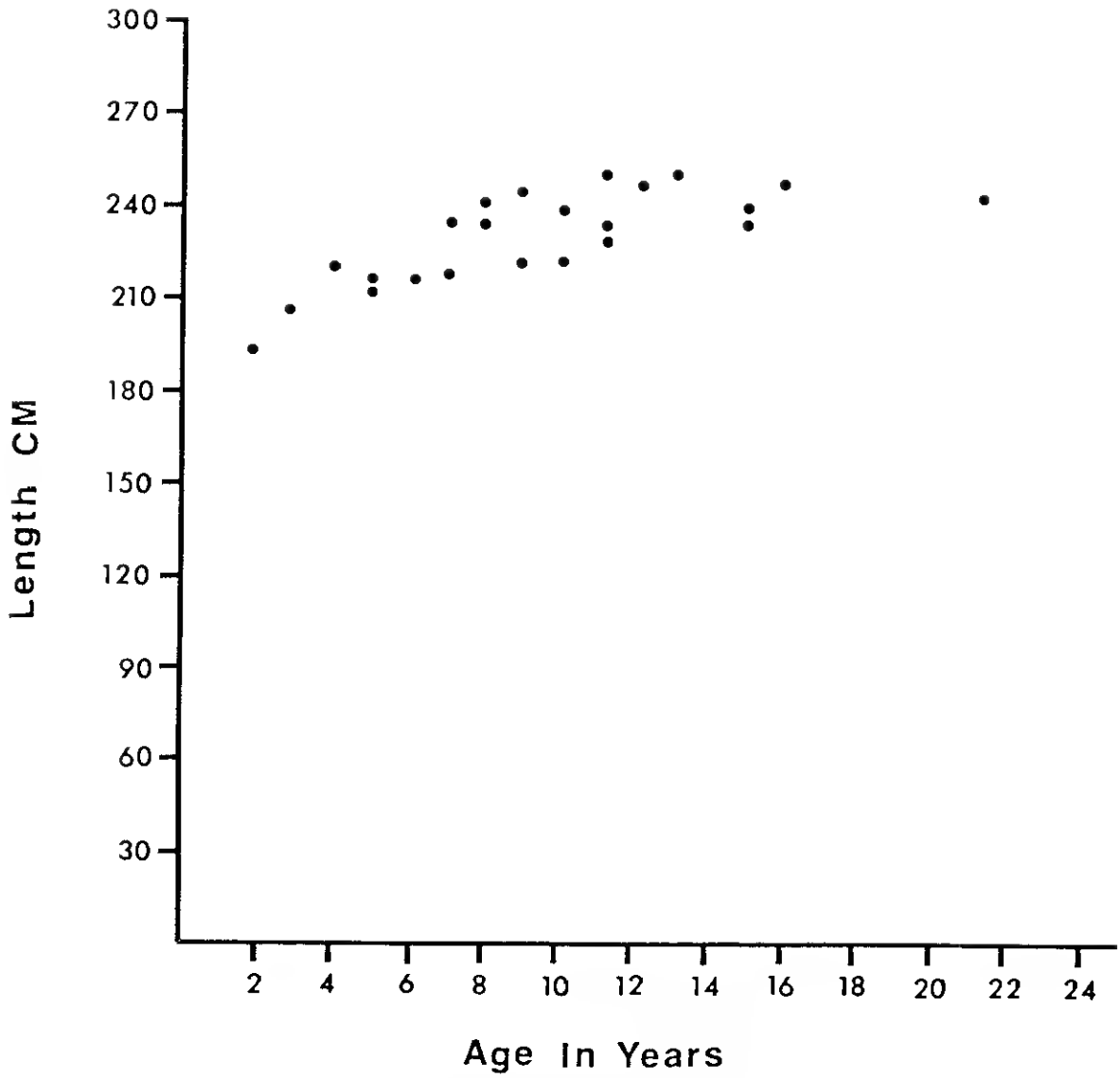


FIGURE 3: LENGTH-AGE RELATIONSHIP FOR FEMALE BOTTLENOSE DOLPHINS COLLECTED FROM MISSISSIPPI SOUND.

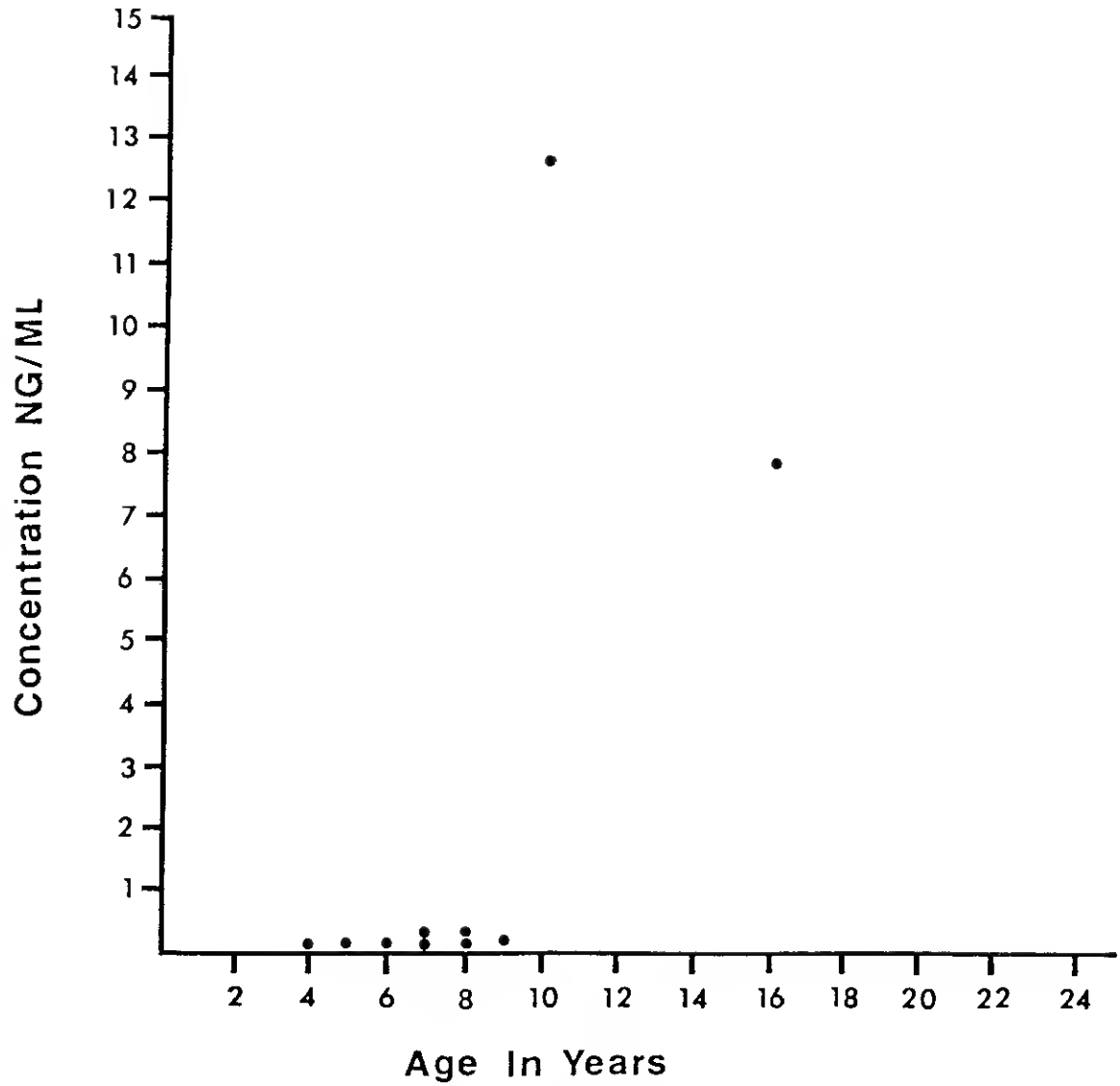


FIGURE 4: SERUM TESTOSTERONE CONCENTRATION AS A FUNCTION OF AGE FOR MALE BOTTLENOSE DOLPHINS FROM THE MISSISSIPPI SOUND.

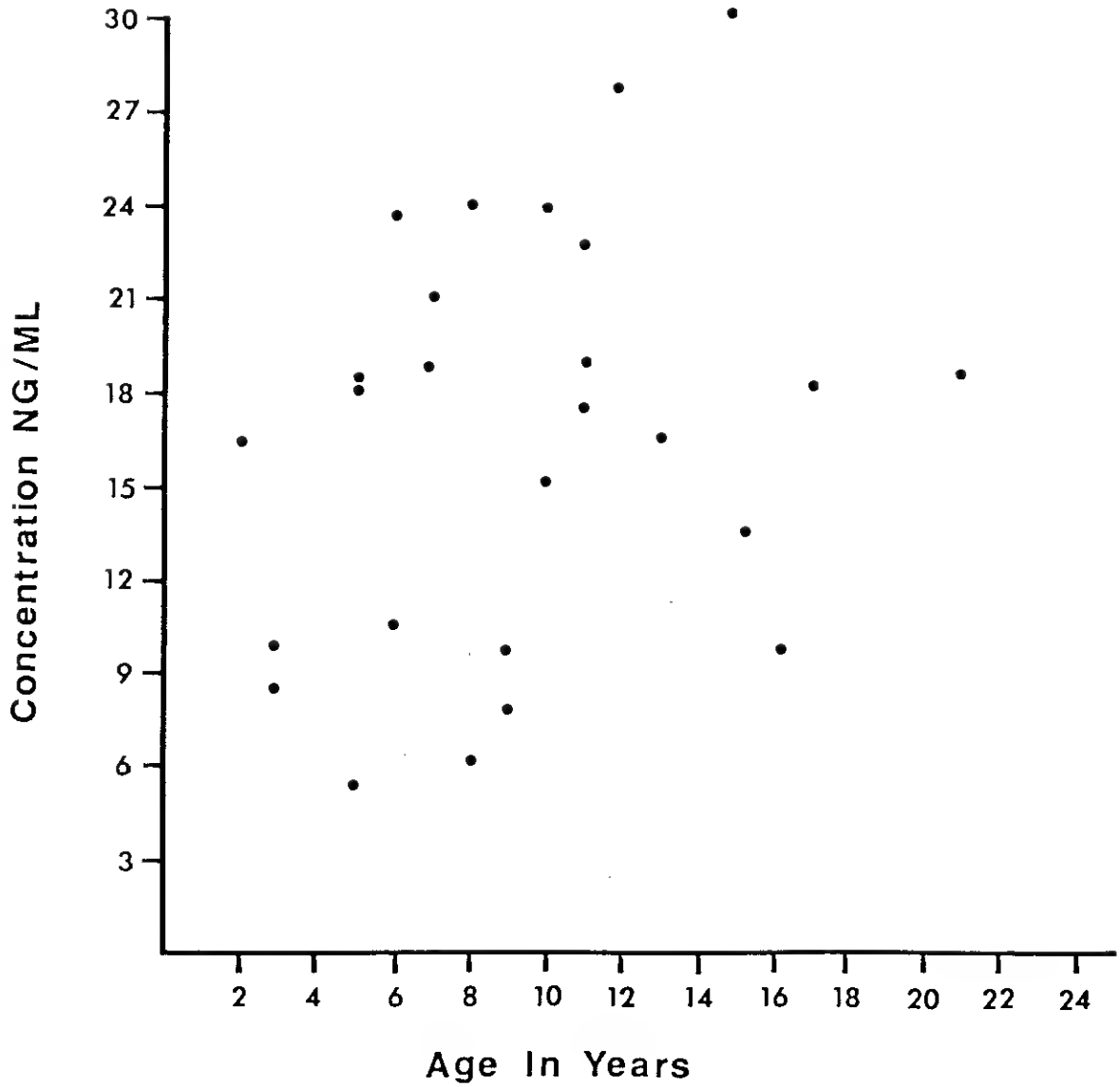


FIGURE 5: SERUM ESTRADIAL CONCENTRATION AS A FUNCTION OF AGE FOR FEMALE BOTTLENOSE DOLPHINS FROM THE MISSISSIPPI SOUND.

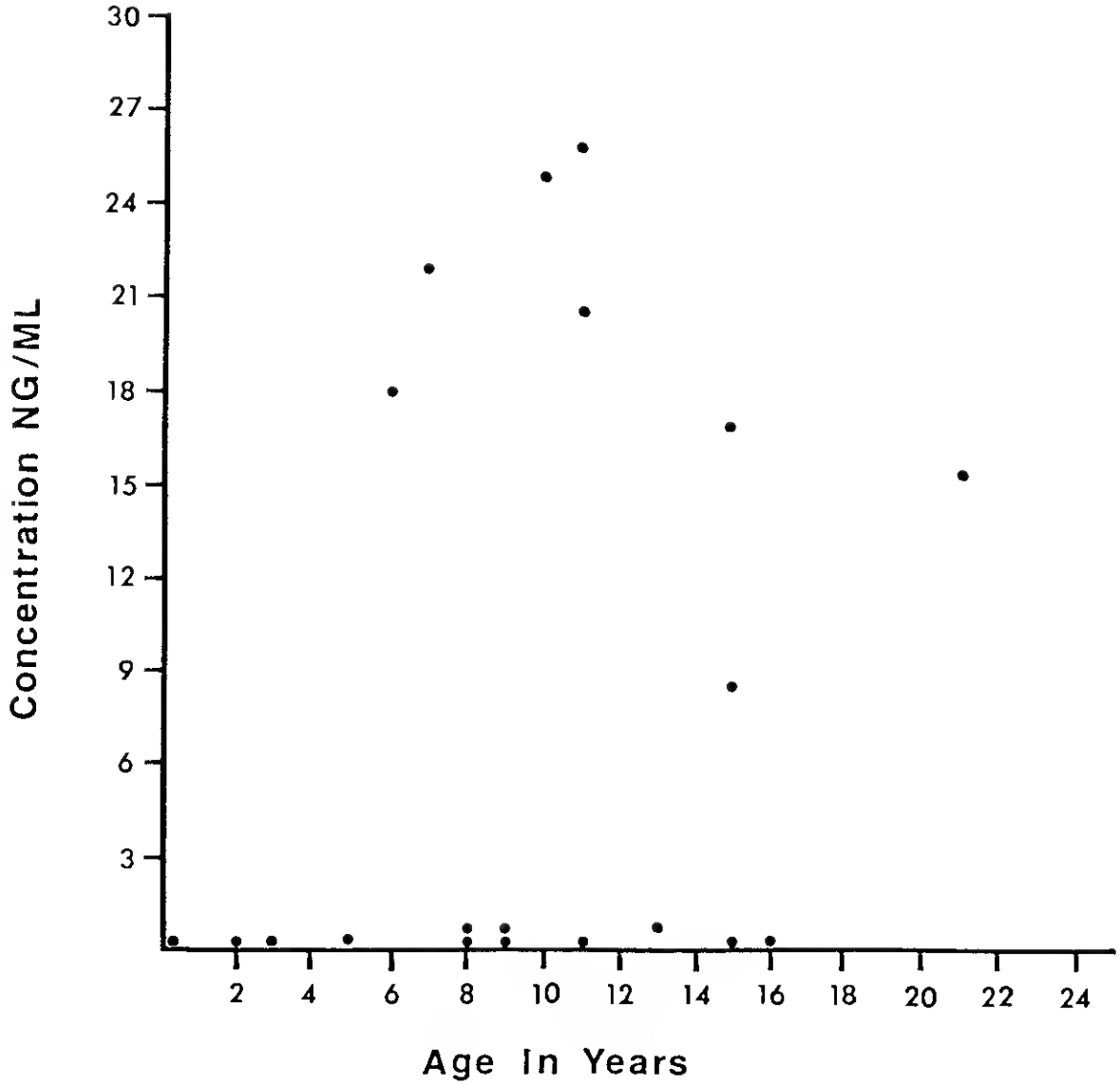


FIGURE 6: SERUM PROGESTERONE CONCENTRATION AS A FUNCTION OF AGE FOR FEMALE BOTTLENOSE DOLPHINS FROM THE MISSISSIPPI SOUND.

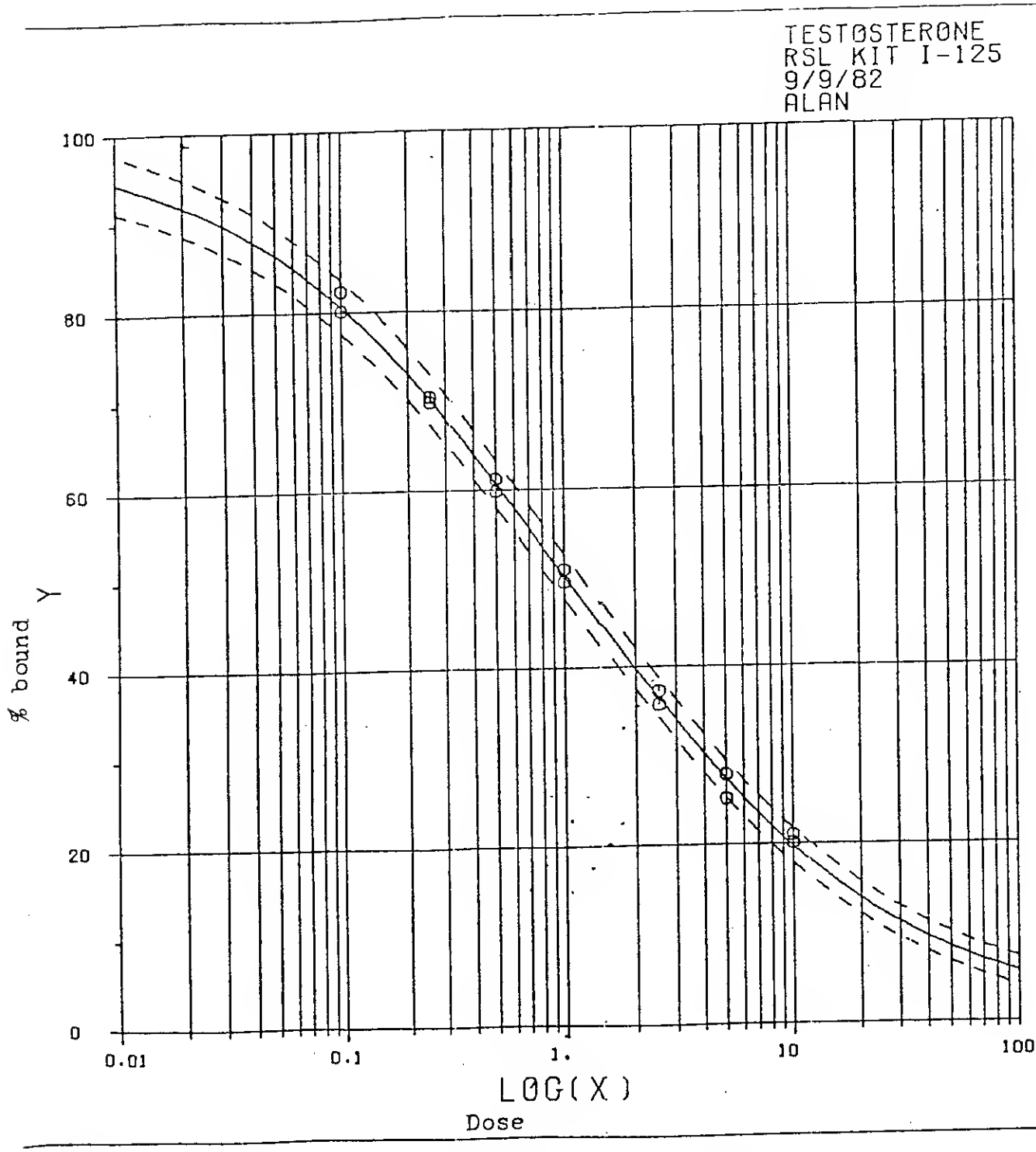


Figure 7: Standard curve generated from the testosterone RIA.

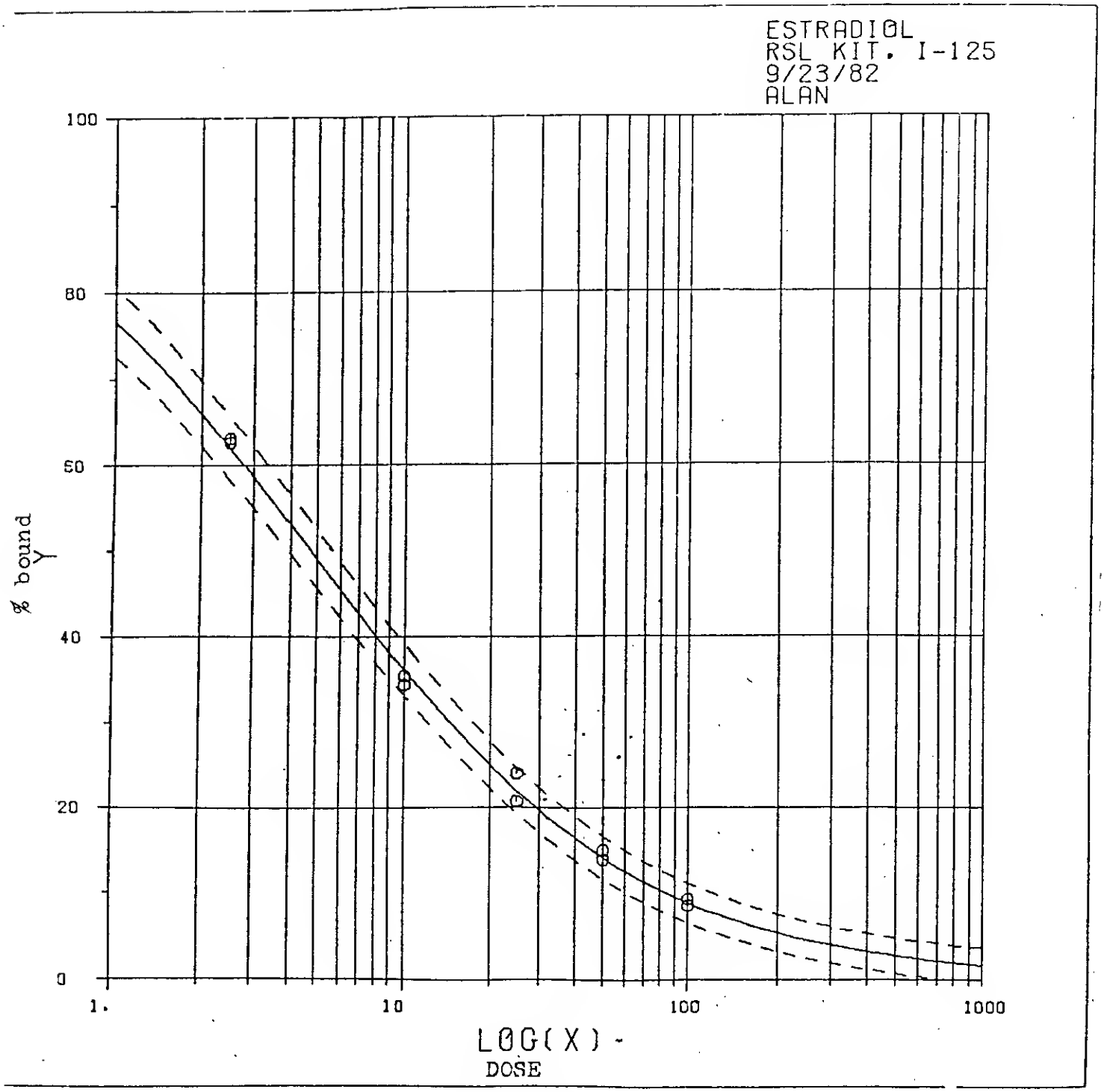


Figure 8: Standard curve generated from the estradiol RIA.

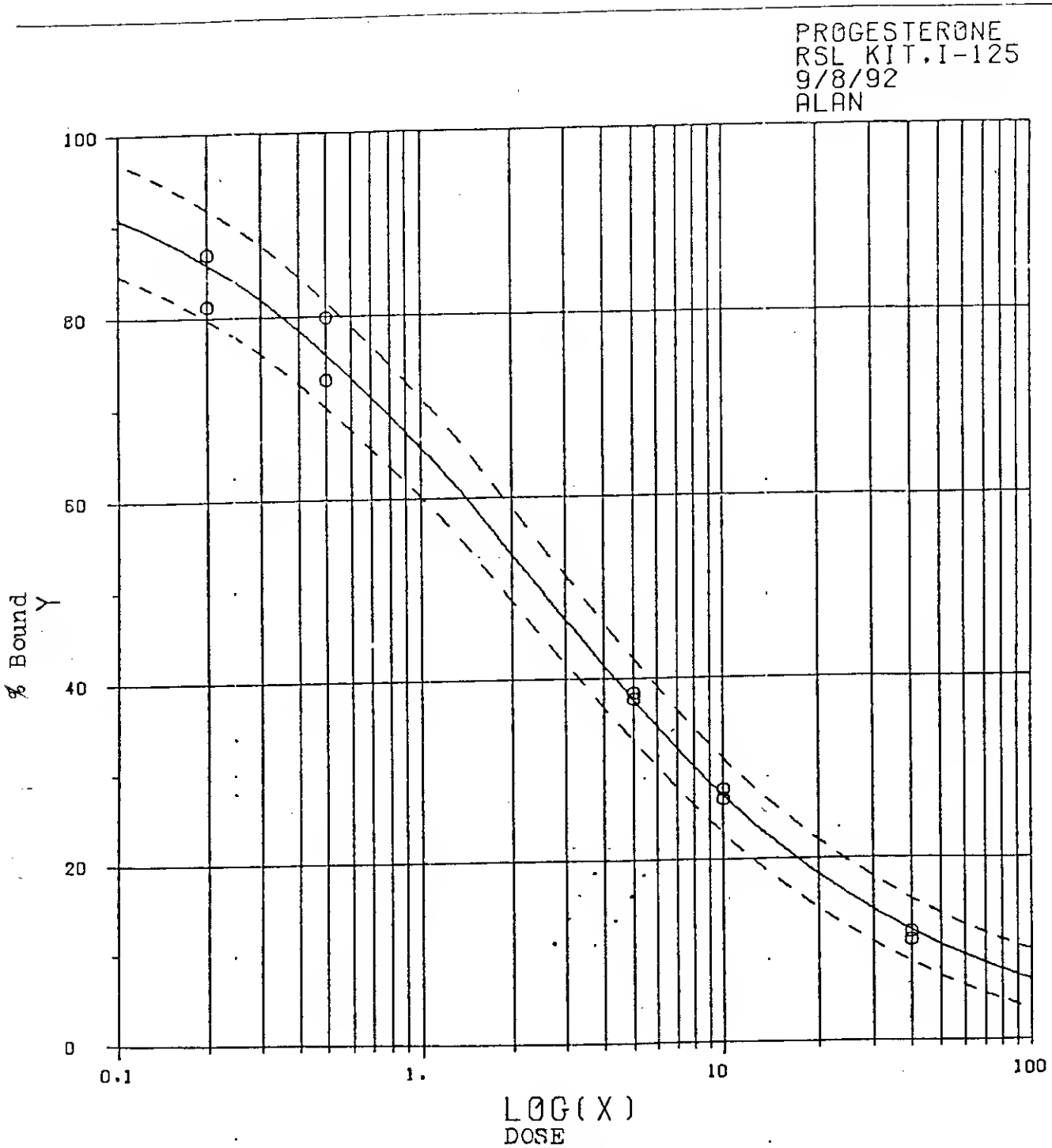


Figure 9: Standard curve generated from the progesterone RIA.

APPENDIX B: Assay Procedures for Endocrinology

ASSAY PROCEDURE FOR ESTRADIOL BY RIA

A. Set up the assay in duplicate in consecutively numbered 10 x 75 mm disposable glass test tubes. (DO NOT USE PLASTIC TUBES). Refer to the Protocol (page 9) as a guide only. Pipet all reagents directly from shipping vials.

B. Extraction of serum or plasma sample:

1. Add 0.1 ml* or 0.6 ml of serum or plasma to an appropriate glass disposable tube.
   NOTE: *This volume to be used only for HMG Ovulation Induction Control samples.

2. Add 6 ml of ethyl acetate: hexane (3:2) (Mallinkrodt catalog numbers 3432 and 4159) to the serum or plasma.

3. Shake or vortex mix vigorously for 60 seconds and allow the phases to separate.

4. Withdraw 5 ml of the organic phase (top phase) and evaporate under air or nitrogen.

5. Reconstitute the sample residue (4) with 2.5 ml of Diluent Buffer and incubate at room temperature for 30 minutes or longer. Swirl or gentle mix during this incubation period.

6. Withdraw duplicate 0.5 ml aliquots for assay.
   NOTE: Each 0.5 ml is equivalent to 0.1 ml serum or plasma if 0.6 ml of sample was extracted, or 0.0166 ml if 0.1 ml of sample was extracted. The dilution factors are 10 and 60 respectively.

C. Assay Conditions:

1. Add 0.6 ml and 0.5 ml of Diluent Buffer to tube numbers 1, 2 and 3, 4, respectively (see Protocol, page 9).

2. Add 0.5 ml (in duplicate) of each Estradiol-17B standard (2.5-100 pg) to tube numbers 5-16.

3. Add 0.5 ml (in duplicate) of reconstituted sample (see Section VII., B, (6) to tube numbers 17 to end of assay.

4. With the Exception of tube numbers 1 and 2, add 0.1 ml of Anti-Estradiol-17B to all the assay tubes.

5. Add 0.1 ml of Estradiol-17B-125_1 to all the assay tubes.

6. Mix all the assay tubes, then incubate for 90 minutes at room temperature.

ASSAY PROCEDURE FOR TESTOTERONE BY RIA

A. Set up assay in duplicate, in consecutively numbered 10 x 75 mm disposable glass test tubes (DO NOT USE PLASTIC TUBES). Refer to the Protocol (page 6) as a guide only. Pipet all reagents directly from shipping vials.

B. Assay conditions:

1) Bring reagents to room temperature prior to use.

2) Add 0.5 ml of DILUENT BUFFER to tube numbers 1 and 2.

3) Add 50 ul of the 0.0 ng/ml STANDARD (testosterone free serum) to tubes number 1, 2, 3, and 4.

4) Add 50 ul (in duplicate) of each TESTOSTERONE STANDARD (0.1 ng/ml - 10 ng/ml) to tubes 5-18.

5) Add 50 ul (in duplicate) of control serum, female serum or diluted male serum* to tube numbers 19 to end of assay.

6) Add 0.1 ml of TBGI SOLUTION to all the assay tubes and mix by shaking the test tube rack for 10 seconds.

7) Add 0.5 ml of TESTOSTERONE - 125_1 to all the tubes. WARNING: This 125_1 TRACER MUST BE ADDED BEFORE THE ANTISERUM.

8) With the exception of tubes numbers 1 and 2, add 0.5 ml of ANTI-TESTOSTERONE to all the tubes.

9) Vortex Mix and incubate at 37° C for 120 minutes.

10) After 120 minute incubation (9), add 0.1 ml of SECOND ANTIBODY to all the tubes. Vortex, mix and incubate at 37° C for 60 minutes.

11) After 60 minute incubation (10), centrifuge all assay tubes at 2300-2500 rpm (1000 x g) for 15 minutes. Aspirate or decant the supernatent. (If decanting, blot the rim of test tubes on absorbent paper before turning right side up).

12) Count the precipitate in a gamma counter.

IMPORTANT NOTICE

For successful performance of the assay, strict adherence to the assay sequence given in Section VII. Items (1-12) should be maintained.

*Take 100 ul of male serum and mix with 100 ul of the 0.0 ng/ml STANDARD (testosterone free serum). Mix gently and withdraw 50 ul into the assay tube.

7. After incubation (6), add 0.1 ml of Second Antibody to all the assay tubes and incubate for 60 minutes.

8. After 60 minutes incubation (7), centrifuge all the assay tubes at 2300-2500 rpm (1000 x g) for 15 minutes. Aspirate or decant the supernatant (if decanting, blot the rim of the test tubes on absorbent paper before turning right side up).

9. Count the precipitate in a gamma counter.

ASSAY PROCEDURE FOR PROGESTERONE BY RIA

A. Set up assay in duplicate, in consecutively numbered 10 x 75 mm disposable glass test tubes (DO NOT USE PLASTIC TUBES). Refer to the Protocol (page 6) as a guide only. Pipet all reagents directly from shipping vials.

B. Assay conditions:

(1) Bring reagents to room temperature prior to use. This normally requires approximately one hour.

(2) Add 0.1 ml of the 0.0 ng/ml STANDARD (progesterone free serum) to tubes number 1, 2, 3, and 4.

(3) Add 0.1 ml (in duplicate) of each PROGESTERONE STANDARD (0.2 ng/ml - 40 ng/ml) to tubes 5-16.

(4) Add 0.1 ml (in duplicate) of CONTROL SERUM or PATIENT SERUM or PLASMA* to tube numbers 17 to end of assay.

(5) Add 0.5 of ASSAY BUFFER to tube numbers 1 and 2.

(6) Add 0.5 ml of ANTI-PROGESTERONE to tube numbers 3 to end of assay. Shake the entire rack of tubes for 30 seconds.

(7) Add 0.2 ml of PROGESTERONE 125_1 to all the tubes. Vortex mix and incubate at 37° C for 60 minutes.

(8) After 60 minute incubation (7), add 0.1 ml of SECOND ANITBODY to all the tubes. Vortex mix and incubate at 37° C for 60 minutes.

(9) After 60 minute incubation (8), centrifuge all assay tubes at 2300 - 2500 rpm (1000 x g) for 15 minutes. Aspirate or decant the supernatant. (If decanting, blot the rim of test tubes on absorbent paper before turning right side up).

(10) Count the precipitate in a gamma counter.

IMPORANT NOTICE

For successful performance of the assay, strict adherence to the assay sequence given in section VII, items (1) - (10), should be maintained.

*If patient sample values are greater than 20 ng/ml, the assay should be repeated, diluting the sample 1:10 with the 0.0 ng/ml STANDARD (PROGESTERONE FREE SERUM)

FOLLOW-UP OBSERVATION

Materials and Methods

Even though follow-up observations for marked animals were not required, Marine Animal Productions, Inc. (M.A.P.) at no cost to the U. S. Government and in cooperation with the Pascagoula Laboratory of the National Marine Fisheries Service are conducting resighting studies of tagged animals.

Soon after the completion of this study, several posters and pre-paid mailing cards were printed and distributed to local marinas, bait shops, enforcement agencies, research laboratories, and the news media. In addition, MAP had a telephone number listed for the general public to call collect with resighting information. A copy of these posters and mailing cards are provided in Figures 1 and 2.

Results and Discussion

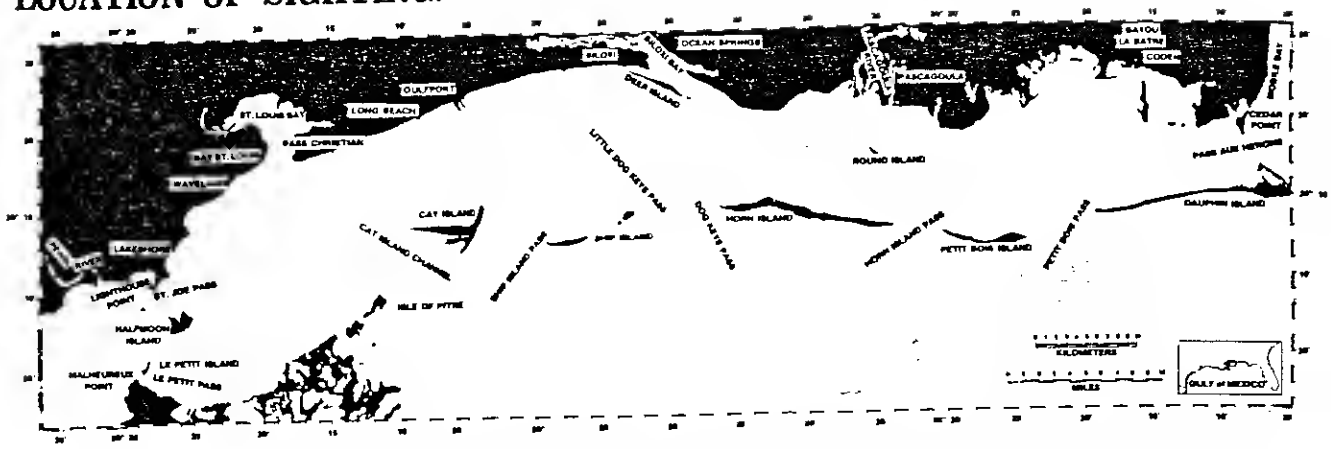
During the study, several previously marked animals were observed and animal numbers 608, 612, 613 were recaptured; however, only animal no. 608 was brought aboard for re-examination. The freeze marks on all resighted animals were clear and the blood and microbiology analysis conducted on animal no. 608 were within the average ranges established for *Tursiops truncatus* in the Mississippi Sound. The results of this re-examination and the large number of resightings of marked animals indicates that the apparent lack of any effect of marking and sampling of studied animals.

At the time of writing of this report, a total of 43 animals had been resighted. Table 1 shows the frequency of each marked animal that had been resighted and Figures 3 to 17 shows the approximate geographical location for each sighting.

TABLE 1. RESIGHTING FREQUENCY OF MARKED ANIMALS

ANIMAL NO.	RESIGHTING FREQUENCY	ANIMAL NO.	RESIGHTING FREQUENCY
601	1	626	4
602	0	627	3
603	6	628	0
604	3	629	2
605	1	630	6
606	1	631	5
607	1	632	10
608	2	633	2
609	1	634	2
610	1	635	3
611	1	636	2
612	3	637	1
613	2	638	2
614	4	639	1
615	0	640	1
616	2	641	0
617	1	642	1
618	1	643	1
619	2	644	0
620	2	645	1
621	3	646	0
622	1	647	1
623	2	648	1
624	4	649	1
625	0	650	3

LOCATION OF SIGHTING:



BOTTLENOSE DOLPHIN RESEARCH PROJECT

Marine Animal Productions-Marine Life
150 Debuys Road
Biloxi, Ms 39531
(601) 896-3981

NATIONAL MARINE FISHERIES SERVICE

MARKED DOLPHIN SIGHTINGS
PHONE COLLECT (601) - 896-3981

LOCATION _____

DATE _____

TIME OF DAY _____

BRAND #'S _____

#ANIMALS IN HERD _____

WEATHER CONDITIONS _____

NAME, ADDDRESS _____

_____

TELEPHONE # _____

REMARKS: _____

_____

_____

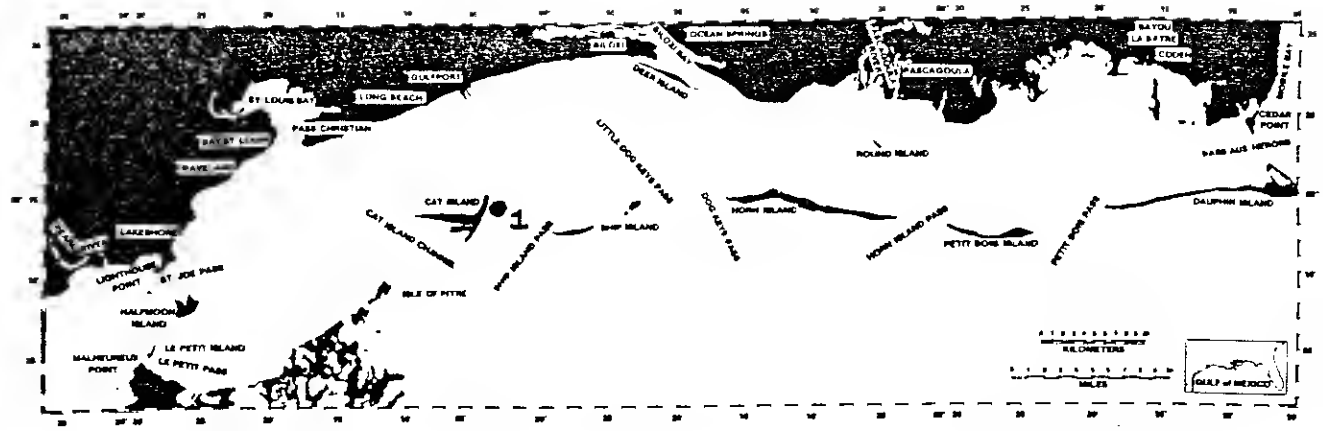
_____

U.S.MAIL

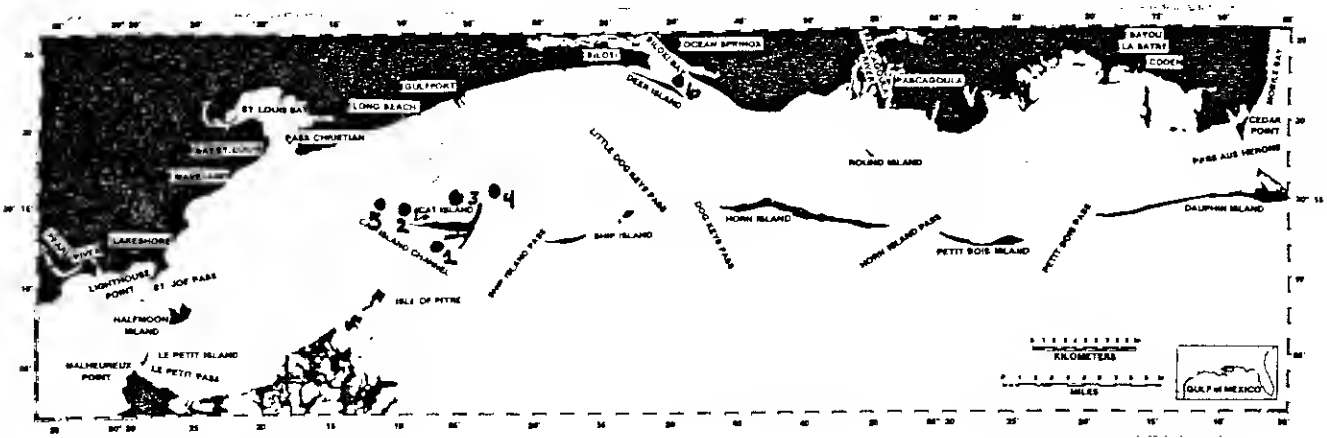
POSTAGE AND FEES PAID
U.S. DEPARTMENT OF COMMERCE
COM 210

Marine Animal Productions, Inc.
Marine Life Oceanarium
150 DeBuys Road
Biloxi, MS 39531

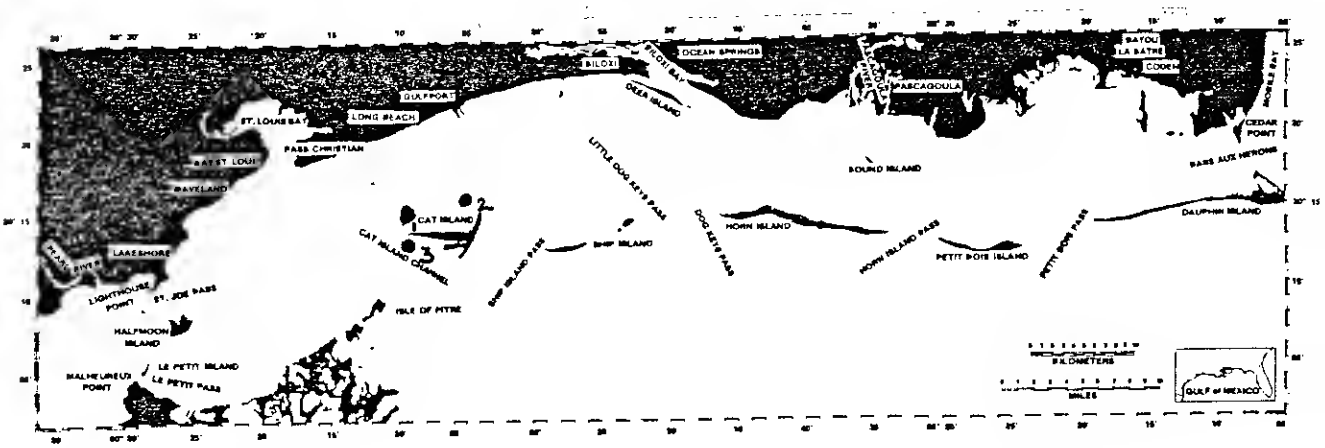
Figure 2: Self-addressed pre-paid post cards used in acquiring resighting information from the public.



Animal No: 601 Sex: F Age: 16

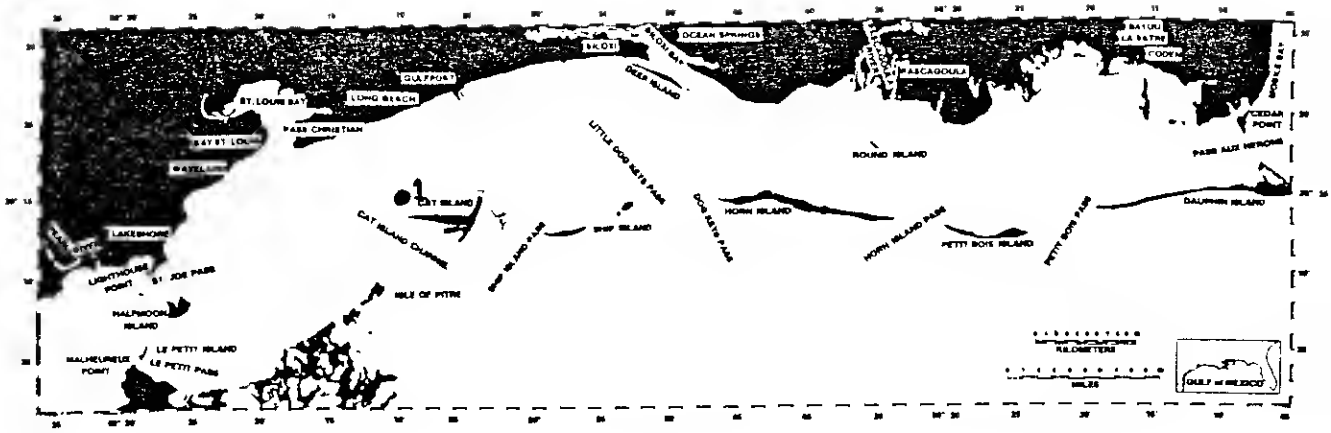


Animal No.: 603 Sex: M Age: 3

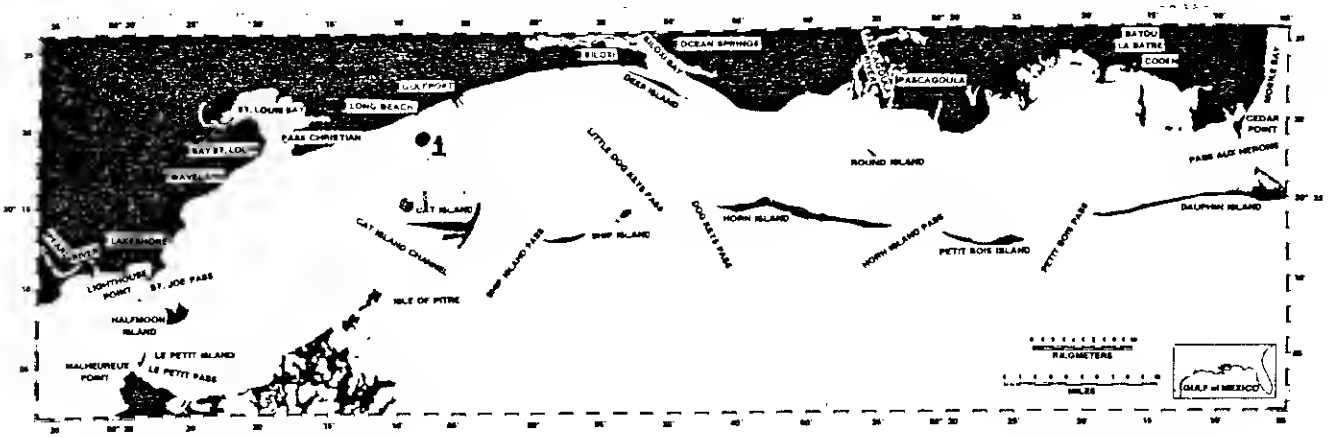


Animal No.: 604 Sex: M Age: 3

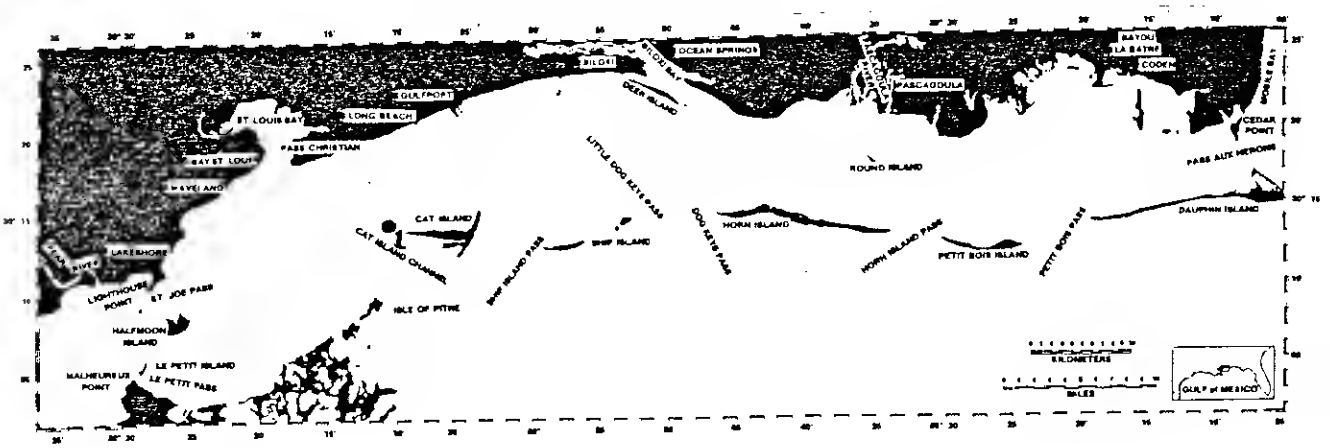
Figure 3: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 605 Sex: F Age: 9

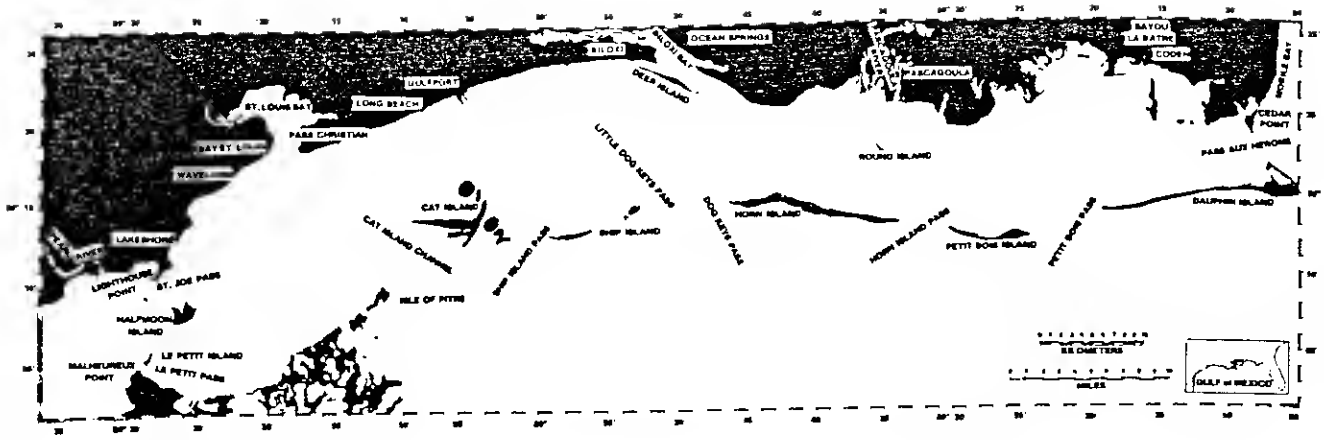


Animal No.: 606 Sex: F Age: 5

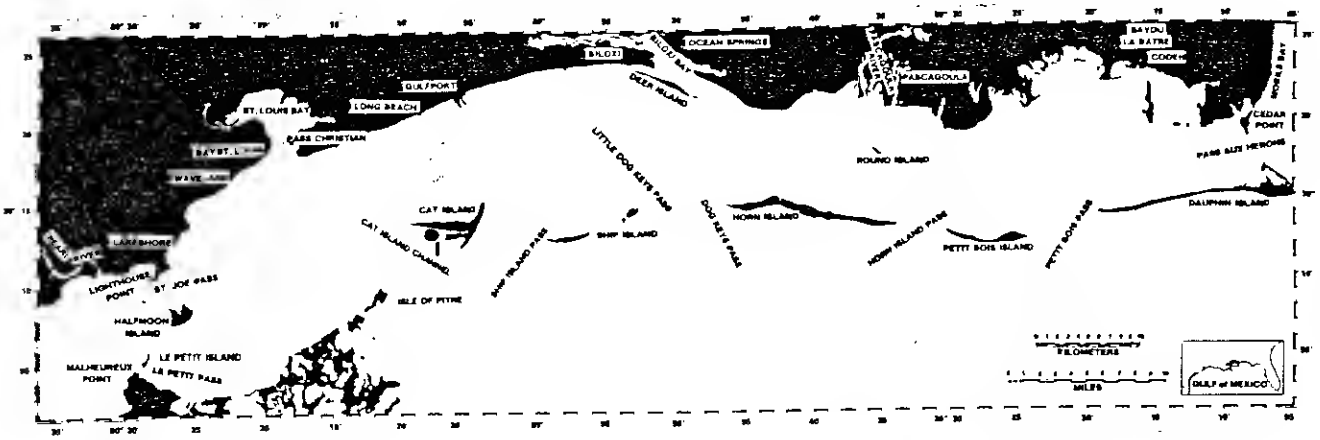


Animal No.: 607 Sex: M Age: 5

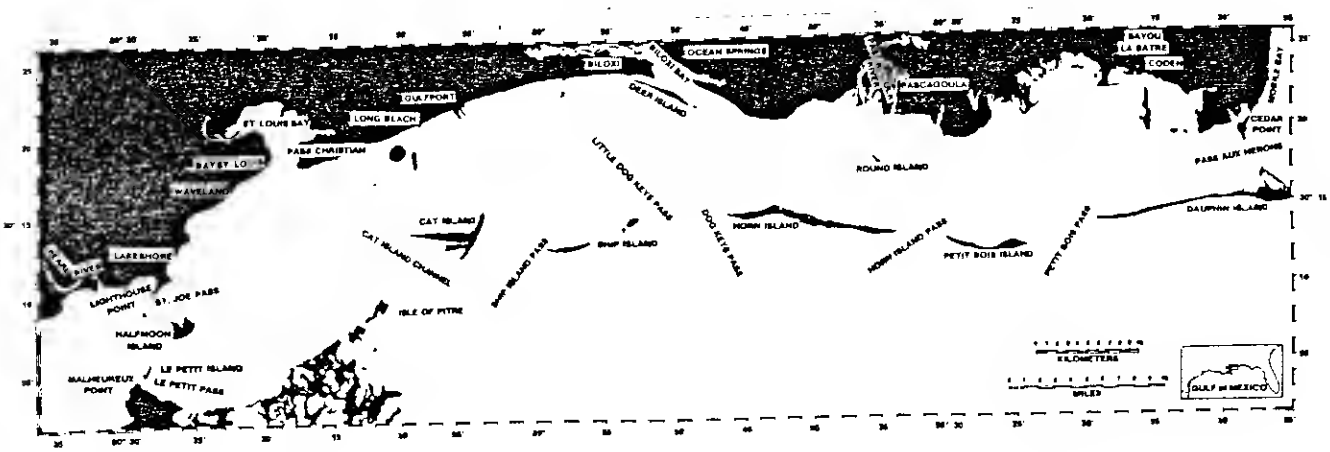
Figure 4: Approximate goegraphical location for individual resighting and frequency of observation for each animal.



Animal No.: 608 Sex: M Age: 9

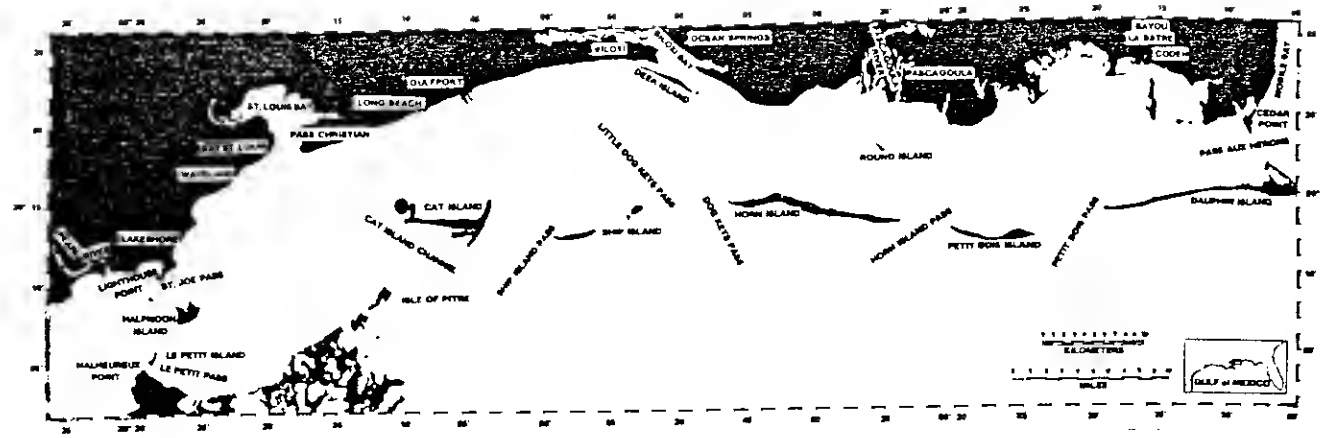


Animal No.: 609 Sex: M Age: 8

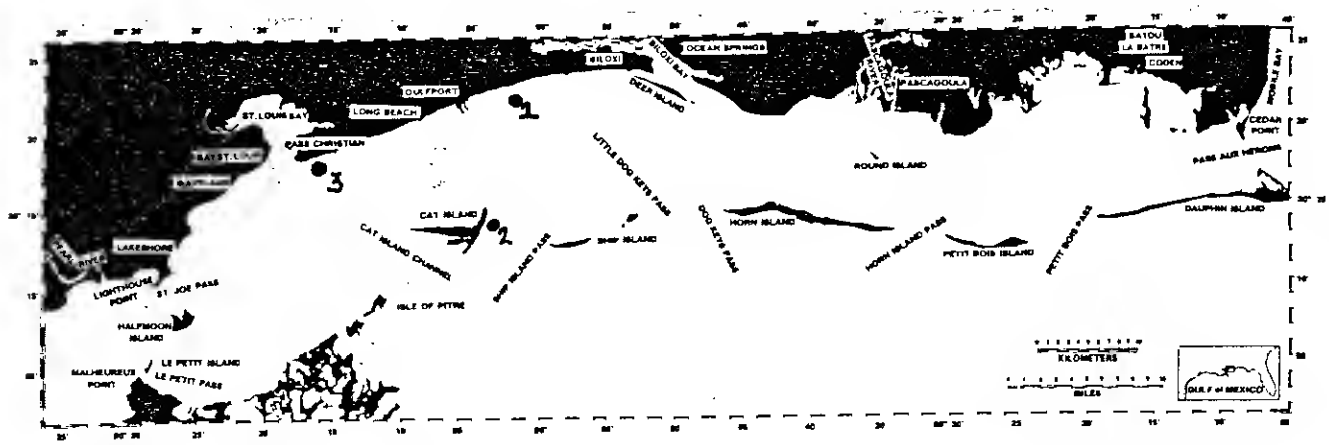


Animal No.: 610 Sex: F Age: 12

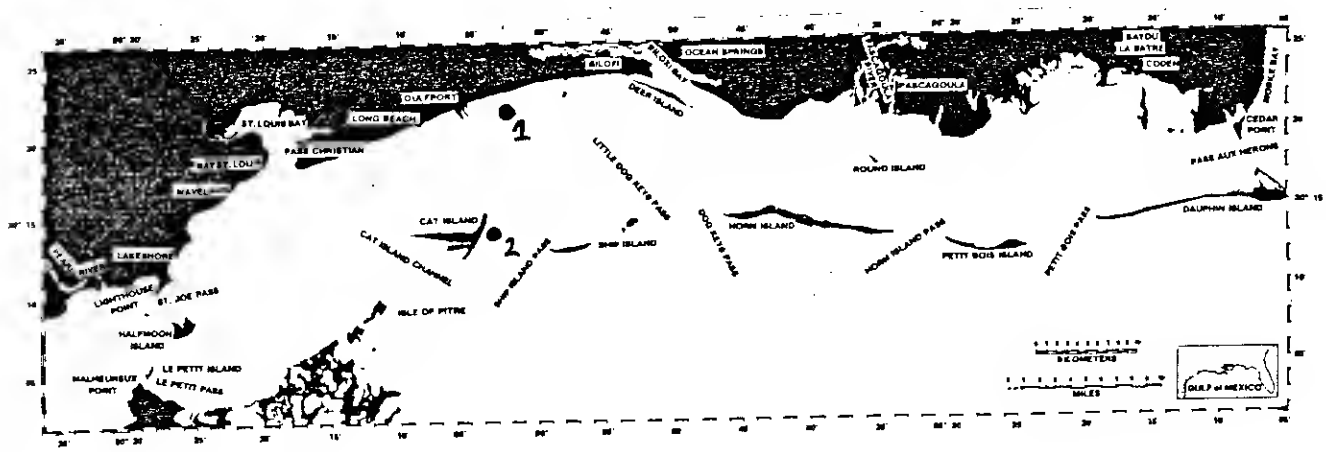
Figure 5: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 611 Sex: F Age: 8+

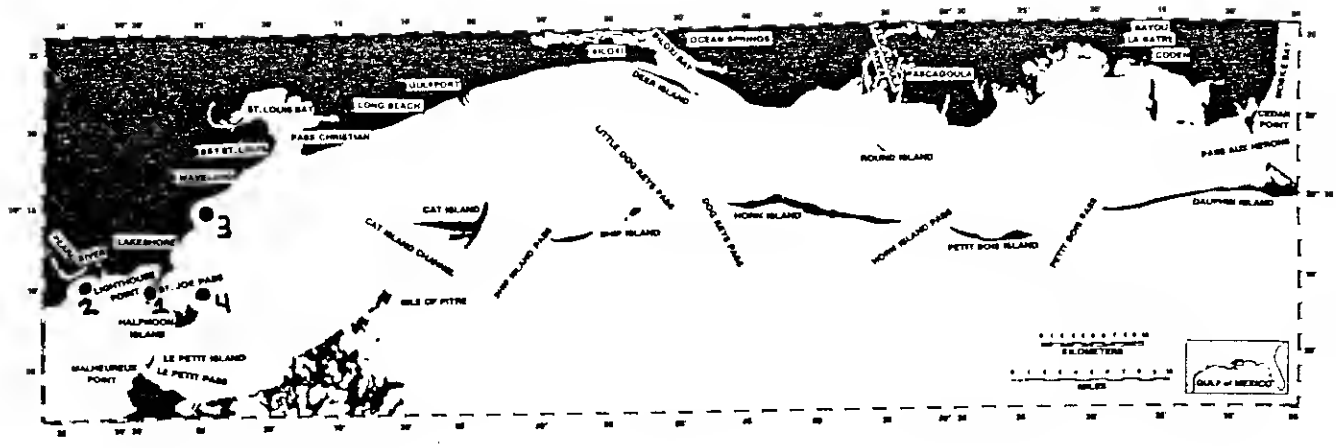


Animal No.: 612 Sex: M Age: 22

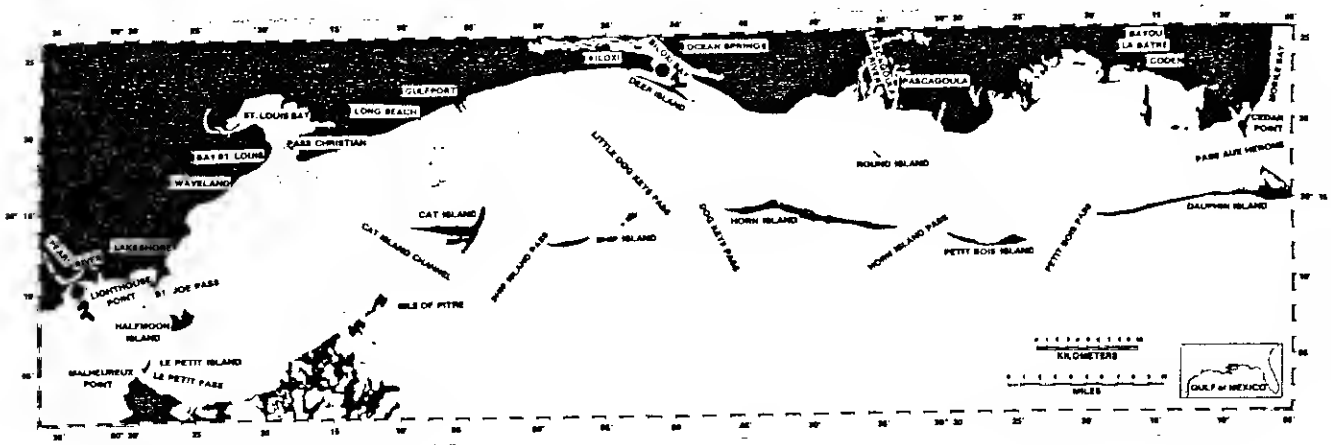


Animal No.: 613 Sex: M Age: 12

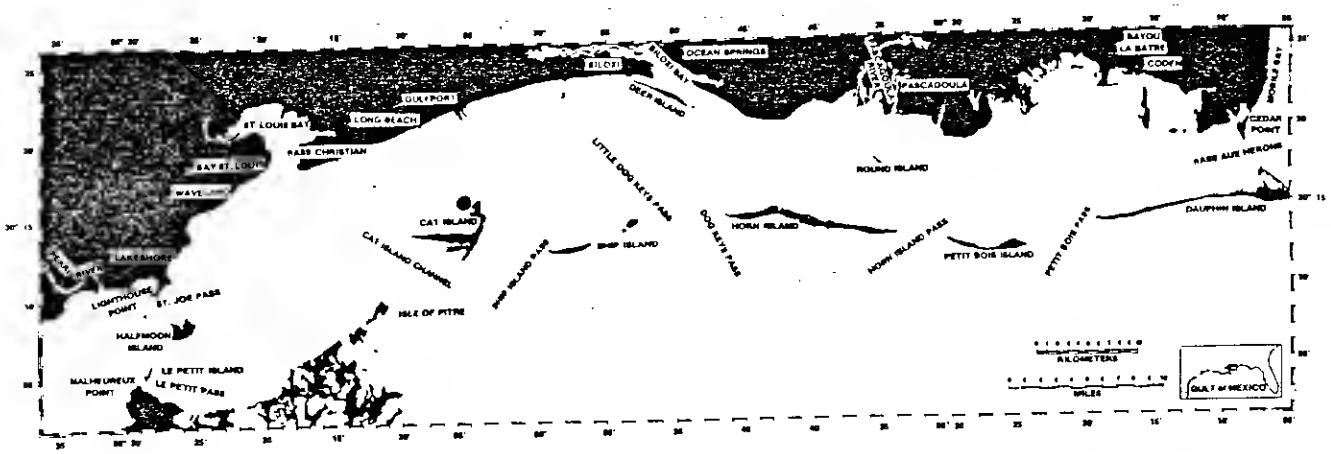
Figure 6: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 614 Sex: F Age: 7

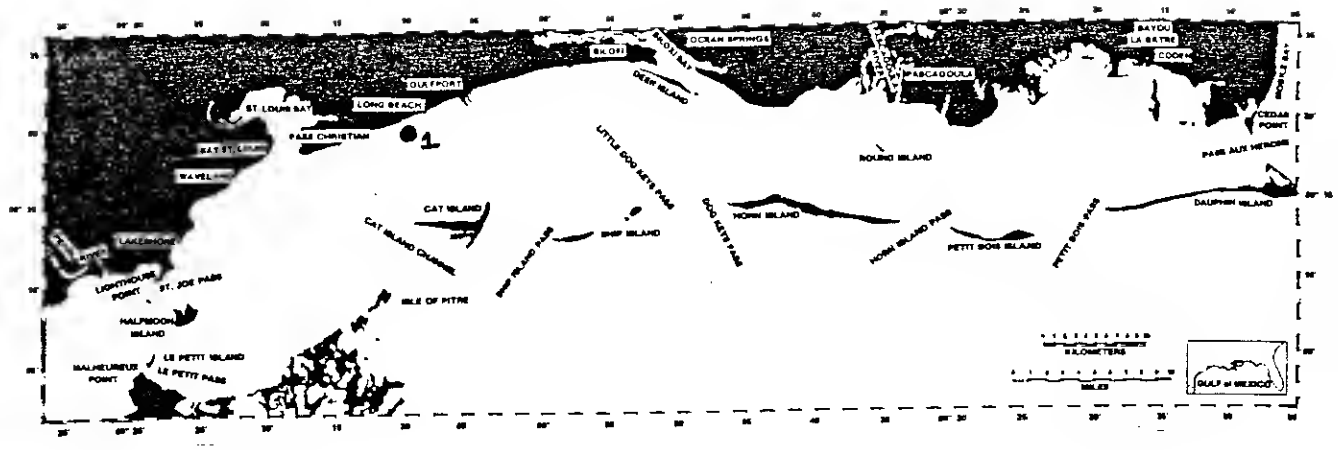


Animal No.: 616 Sex: F Age: 6

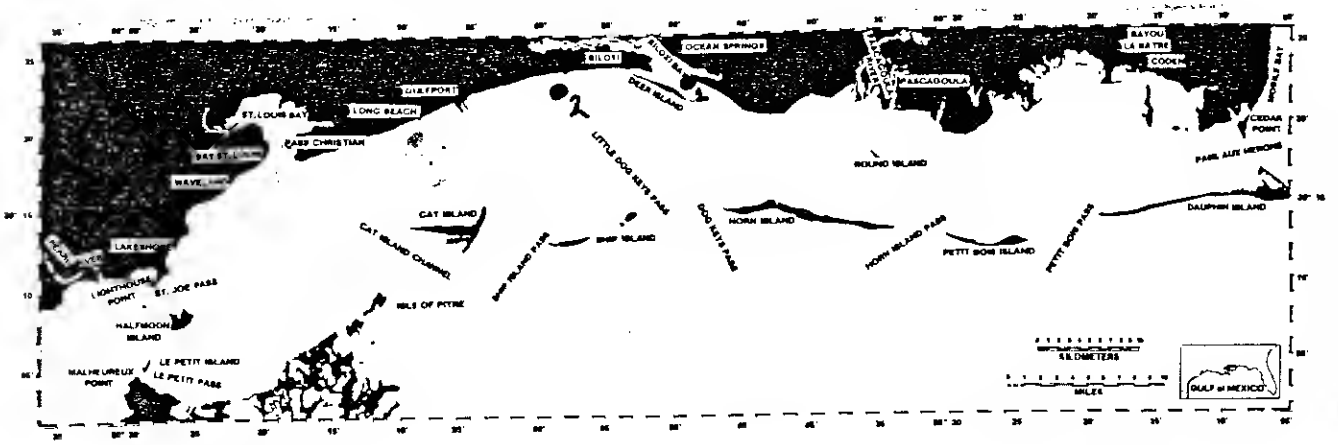


Animal No.: 617 Sex: F Age: Broken tooth

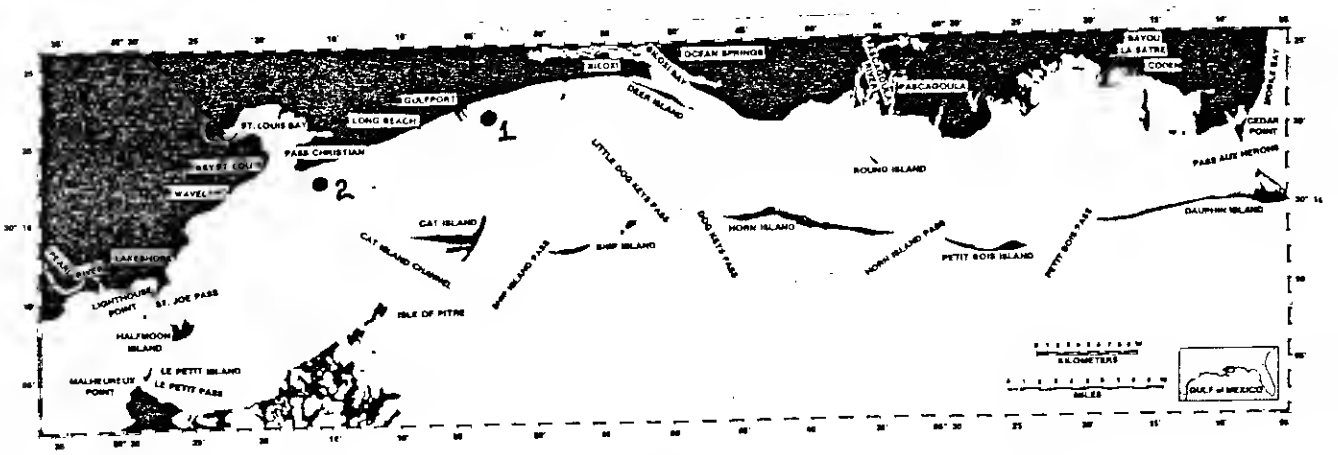
Figure 7: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 618 Sex: F Age: 14

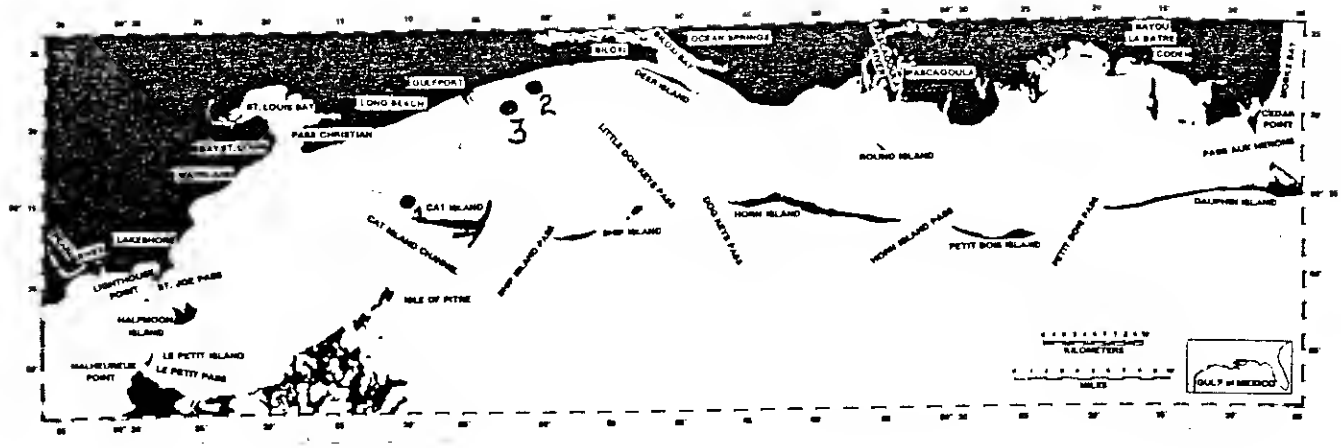


Animal No.: 619 Sex: M Age: 4

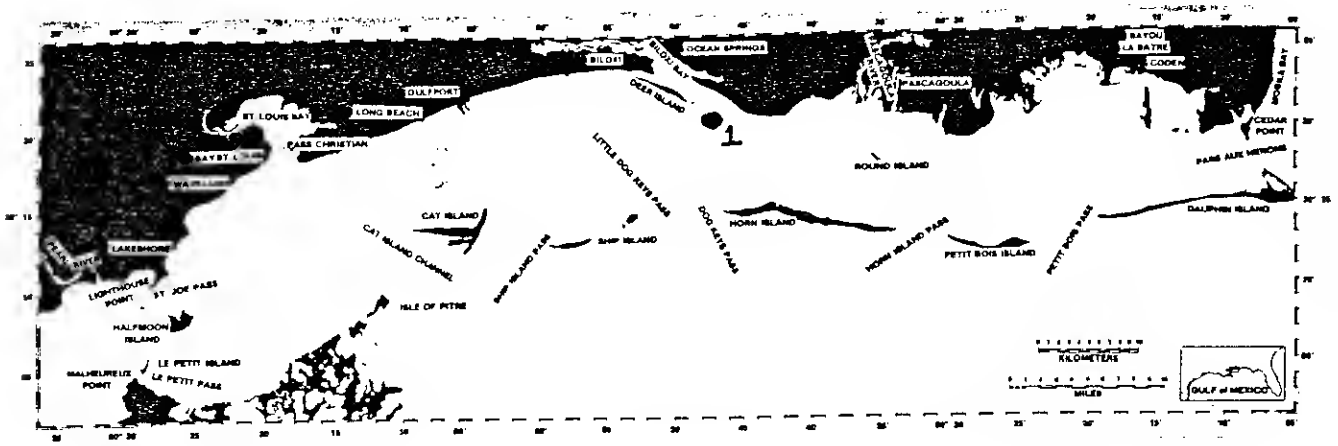


Animal No.: 620 Sex: F Age: 4

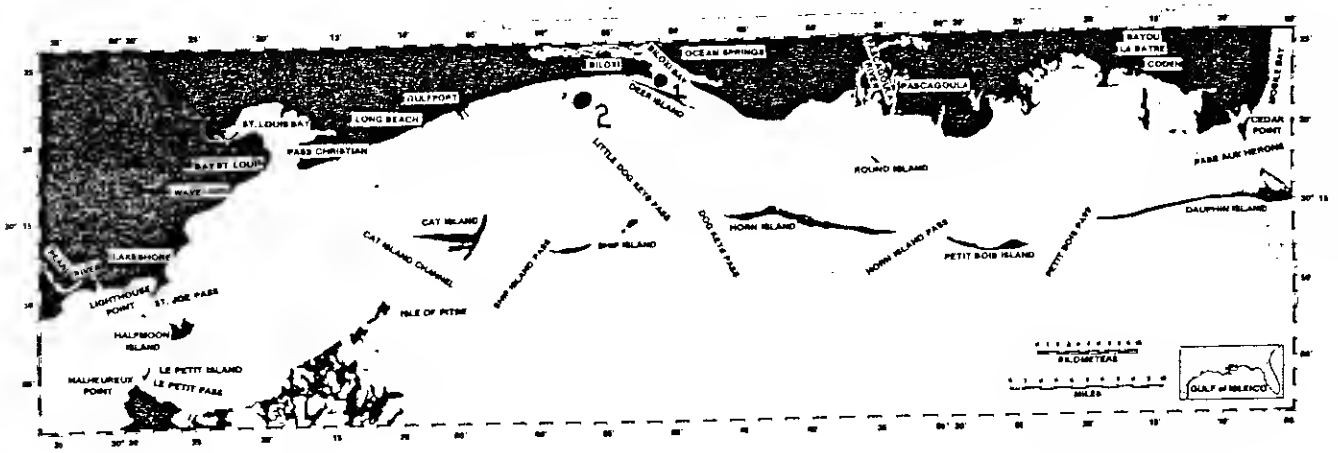
Figure 8: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 621 Sex: M Age: 4

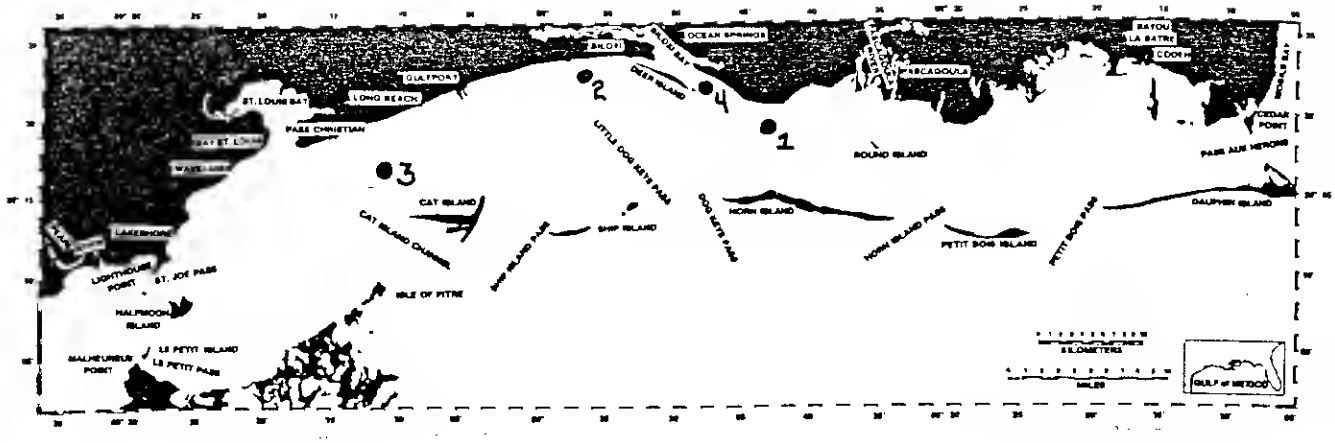


Animal No.: 622 Sex: M Age: 6

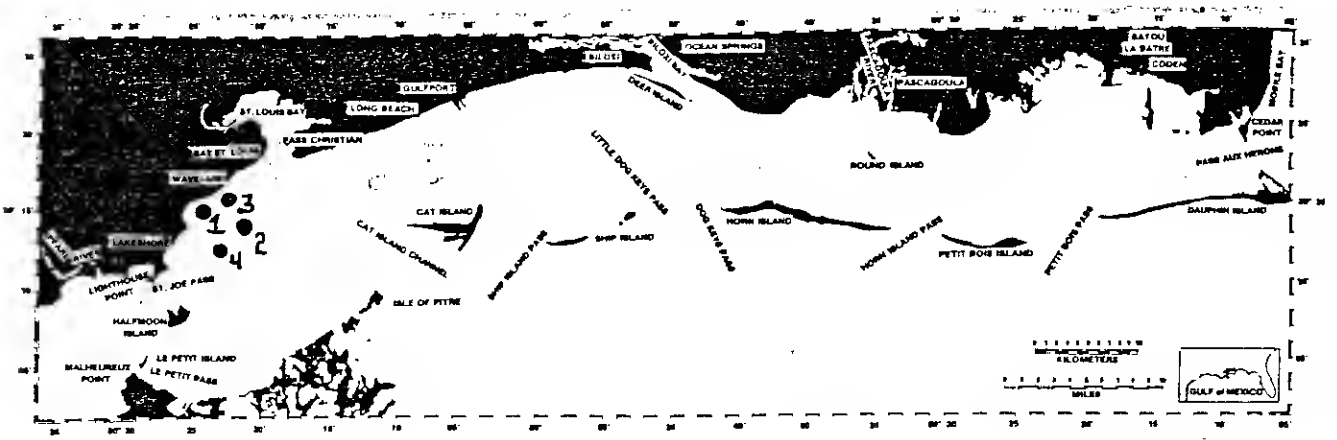


Animal No.: 623 Sex: F Age: Not Determined

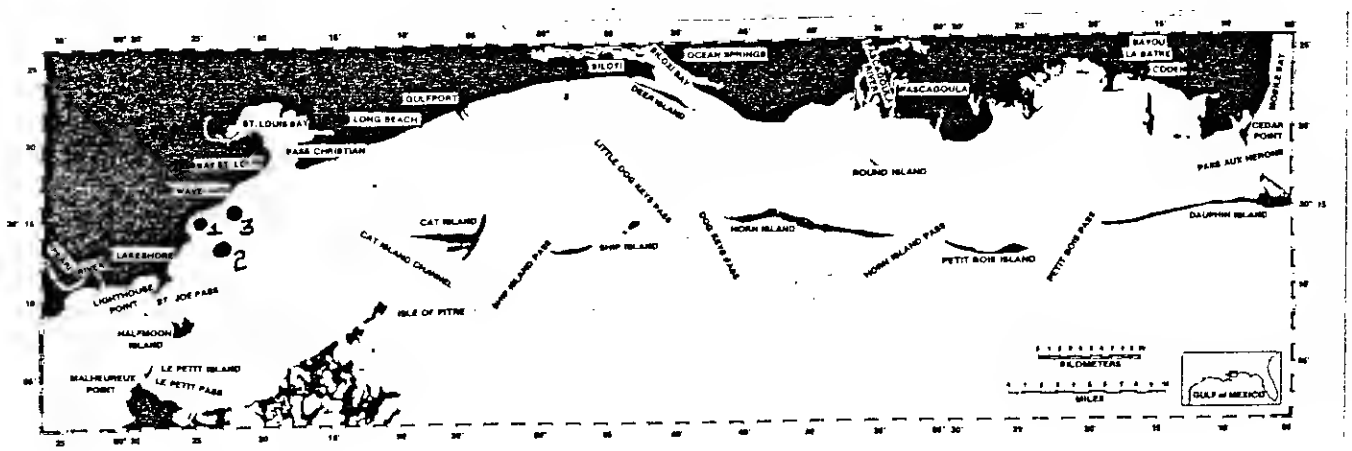
Figure 9: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 624 Sex: M Age: 7

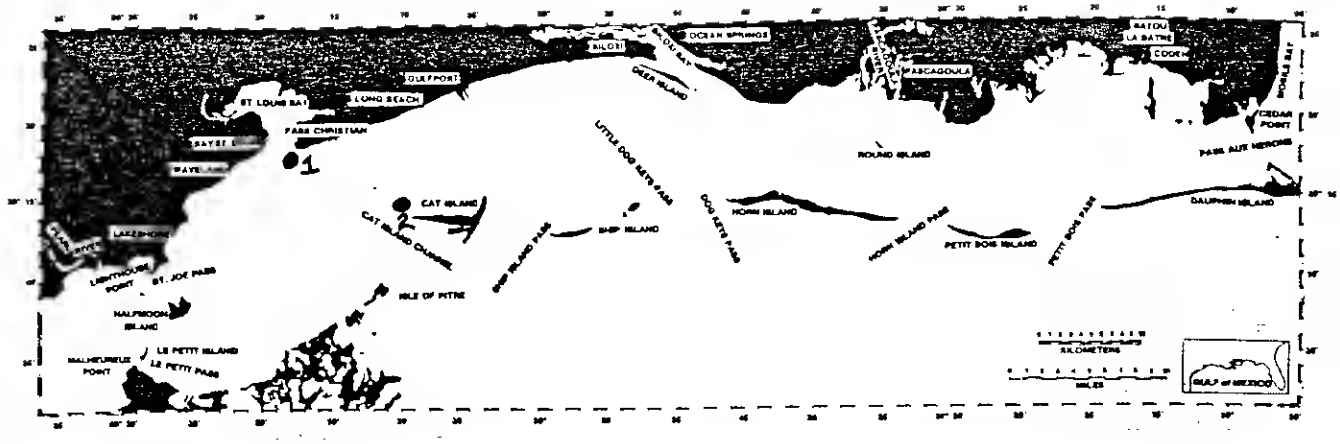


Animal No.: 626 Sex: F Age: 2

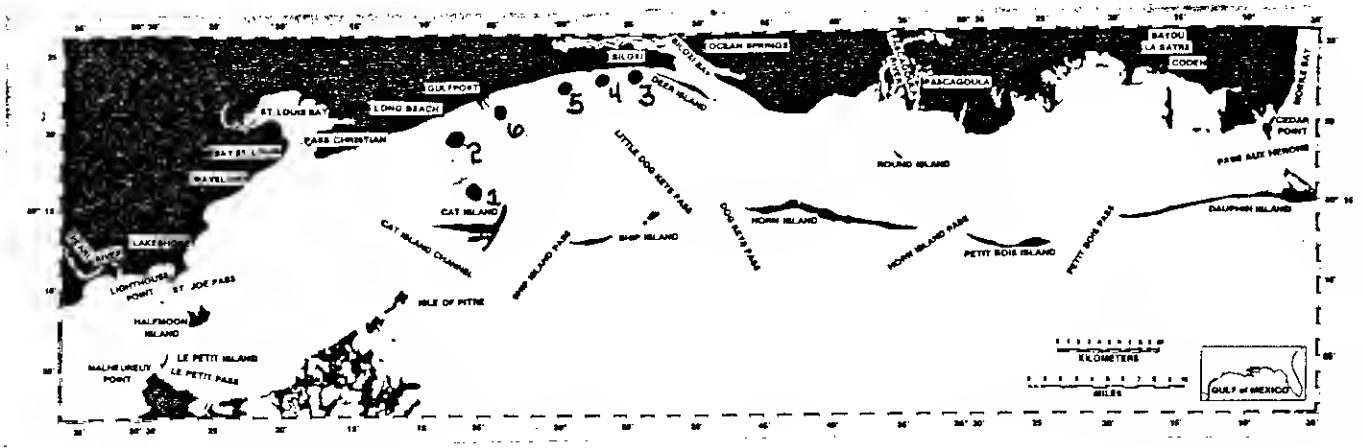


Animal No.: 627 Sex: F Age: 10

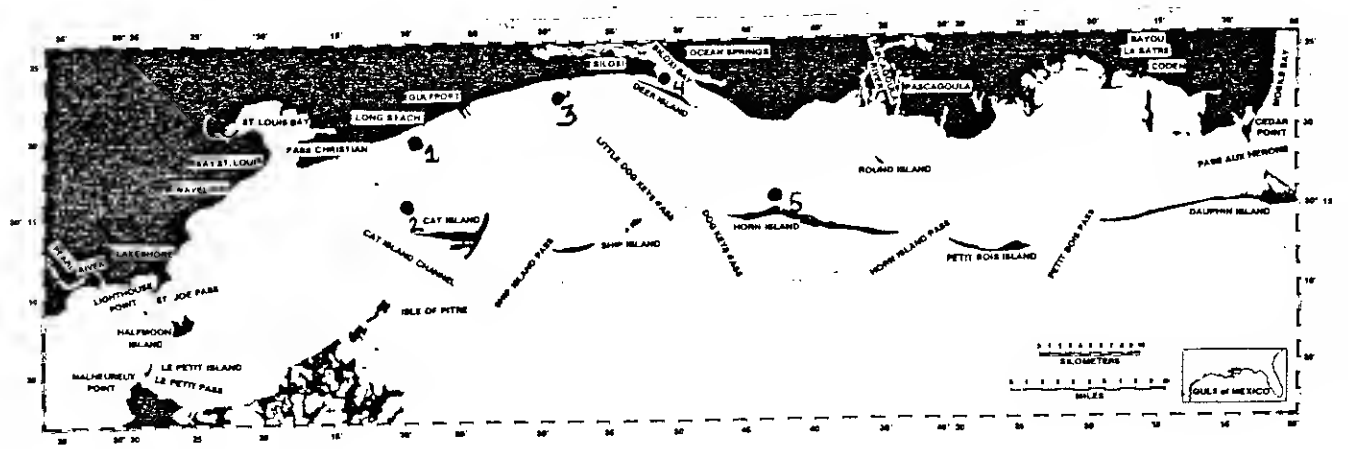
Figure 10: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 629 Sex: F Age: 6

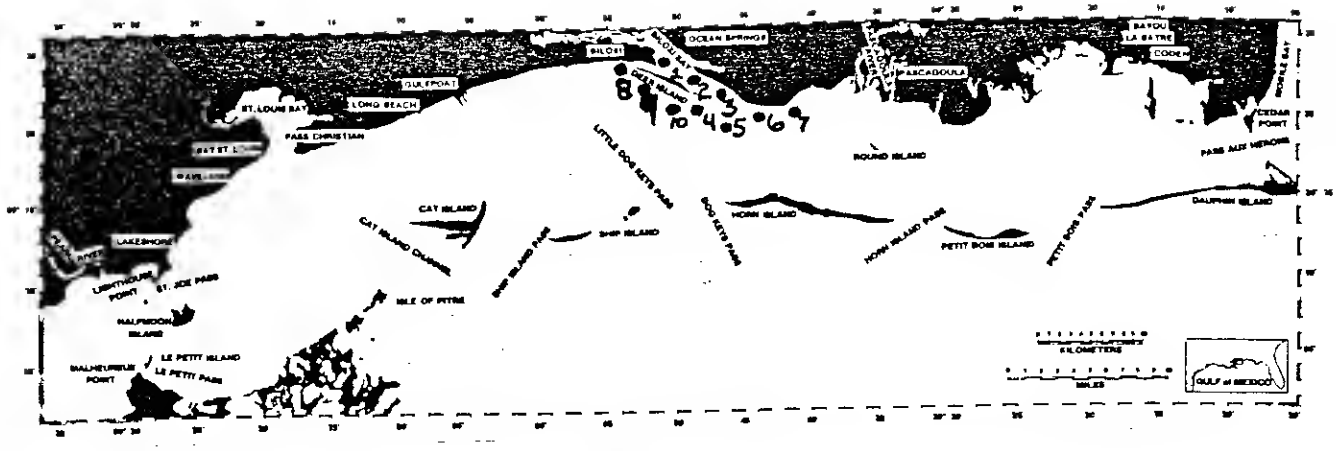


Animal No.: 630 Sex: M Age: 4

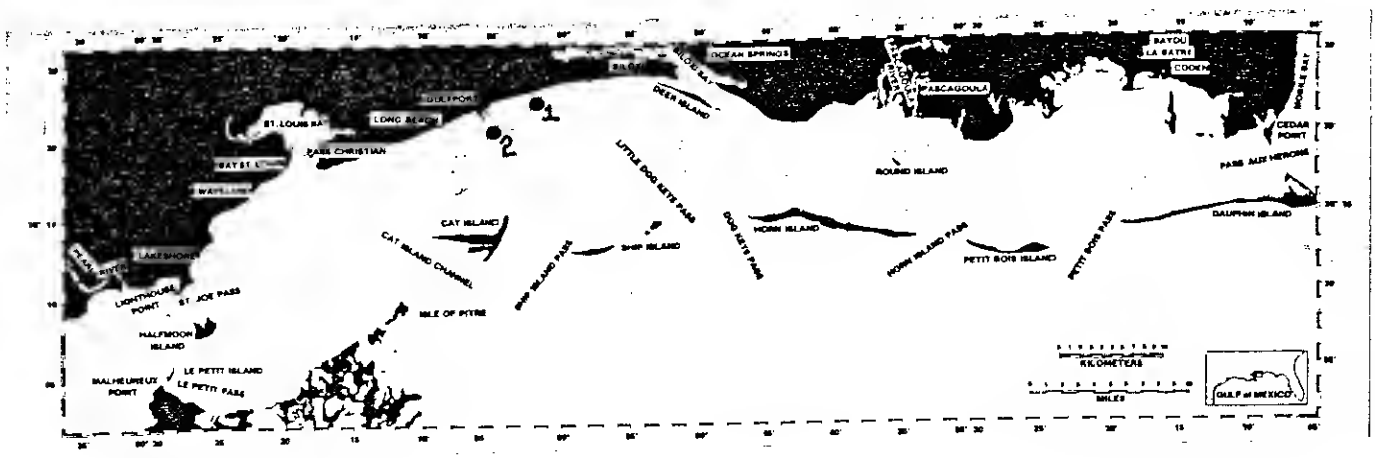


Animal No.: 631 Sex: M Age: 4

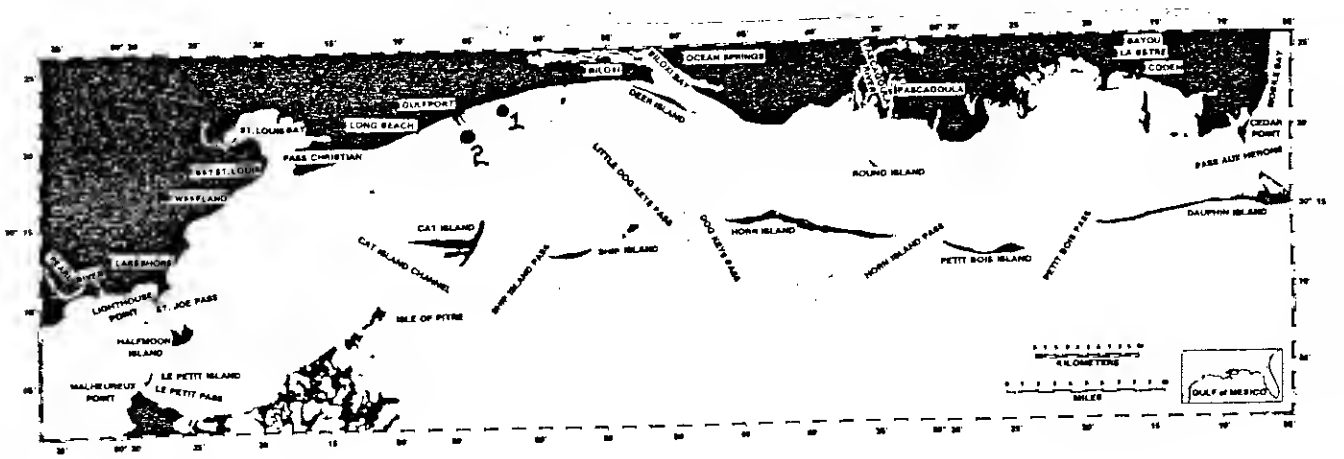
Figure 11: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 632 Sex: F Age: 7

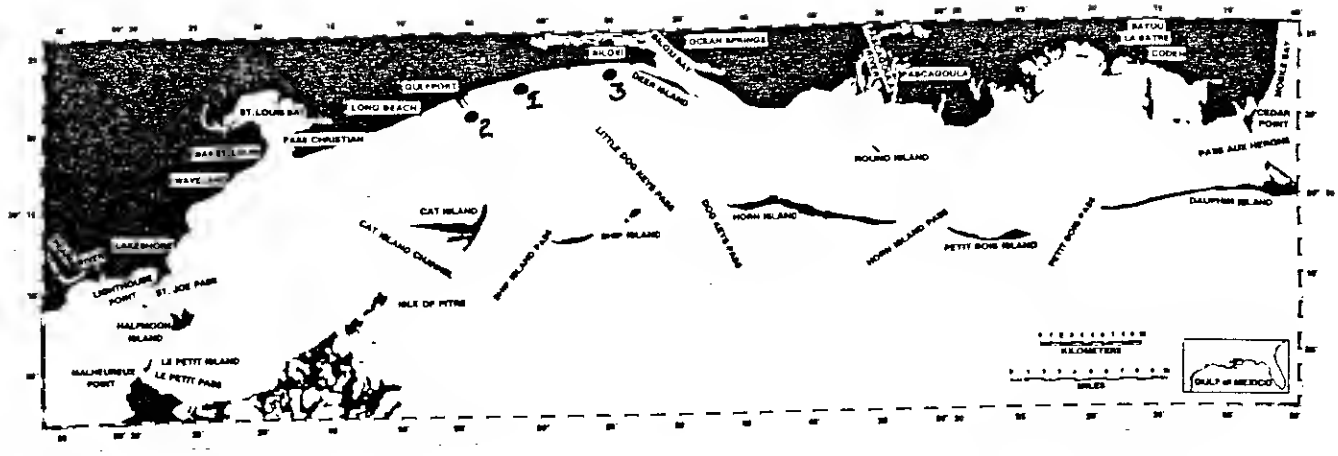


Animal No.: 633 Sex: M Age: 16

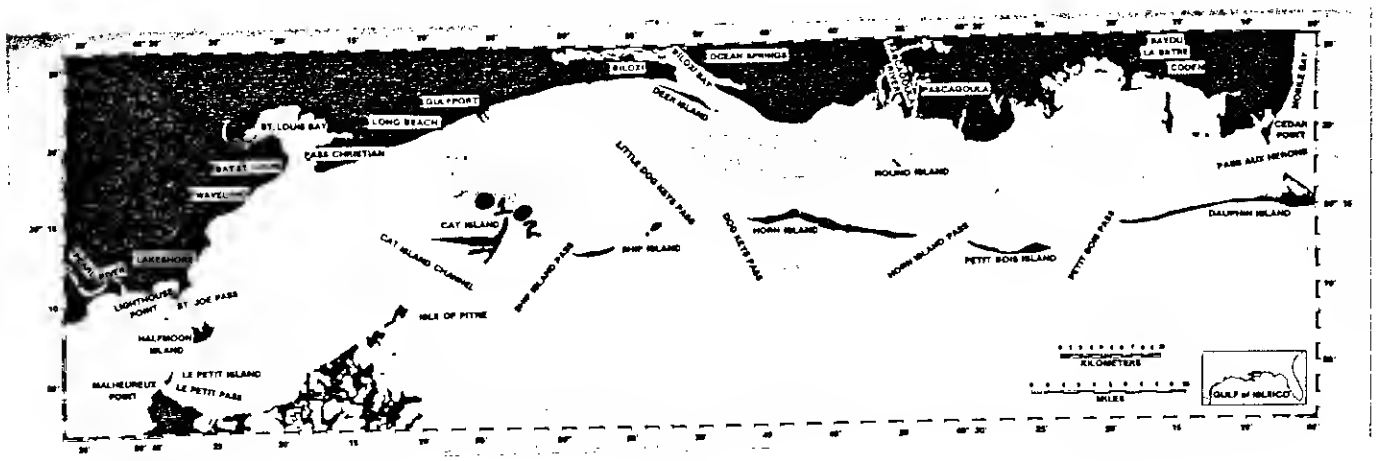


Animal No.: 634 Sex: F Age: 11

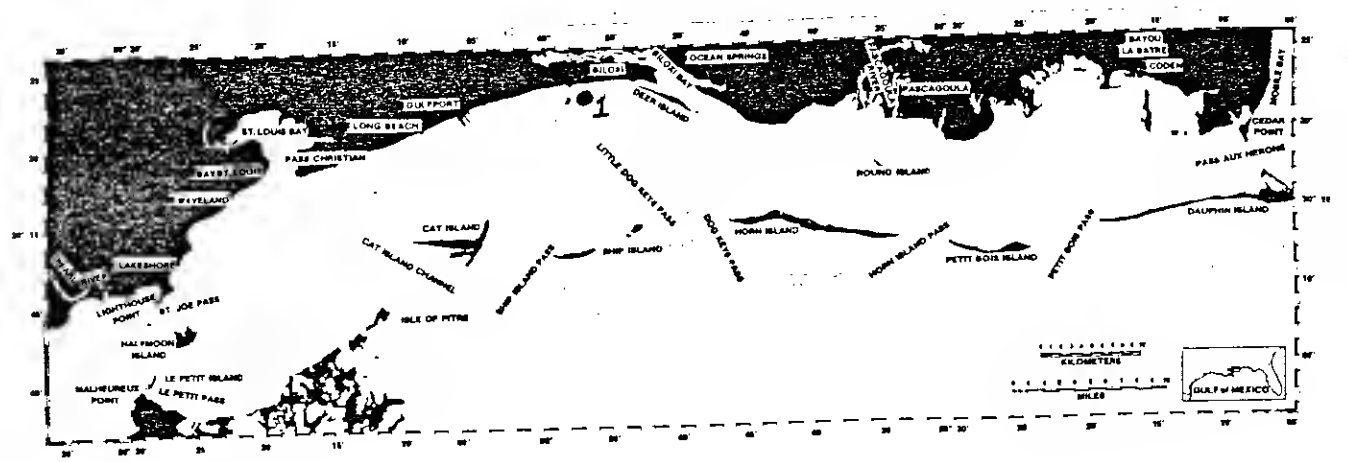
Figure 12: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 635 Sex: M Age: Not Determined

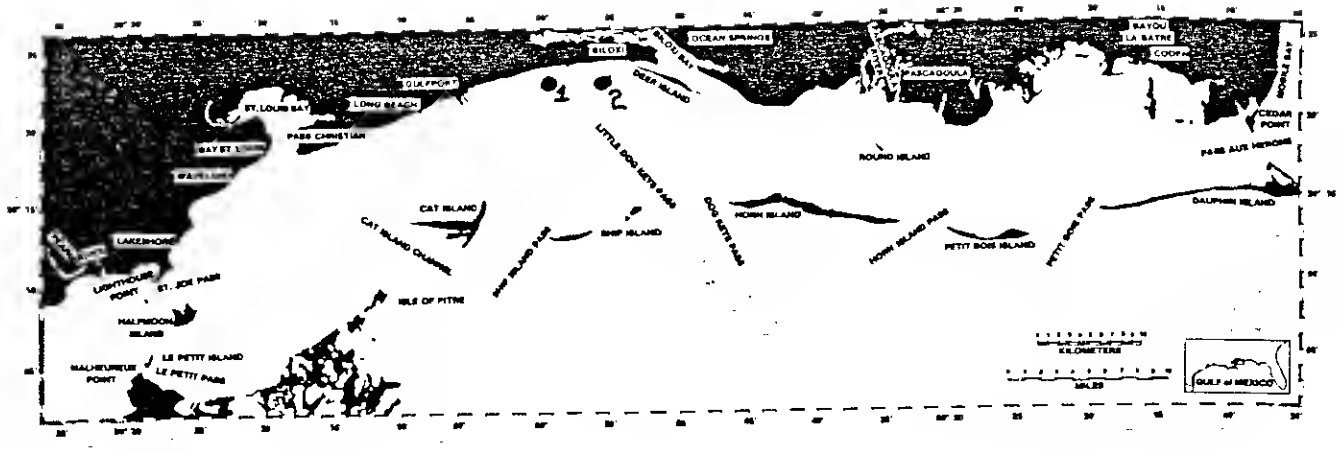


Animal No.: 636 Sex: M Age: 7

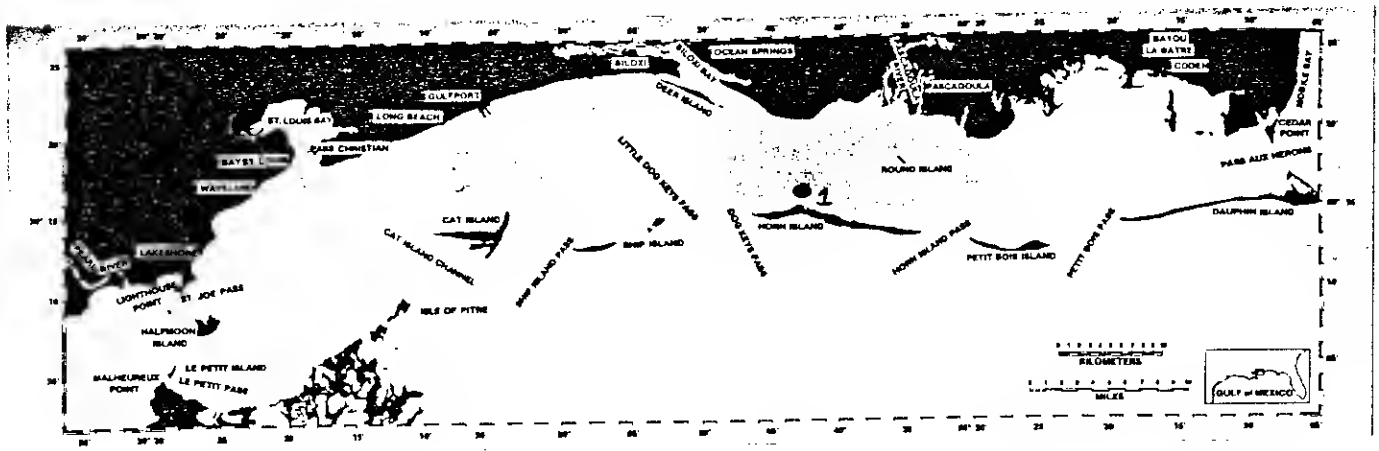


Animal No.: 637 Sex: F Age: 11

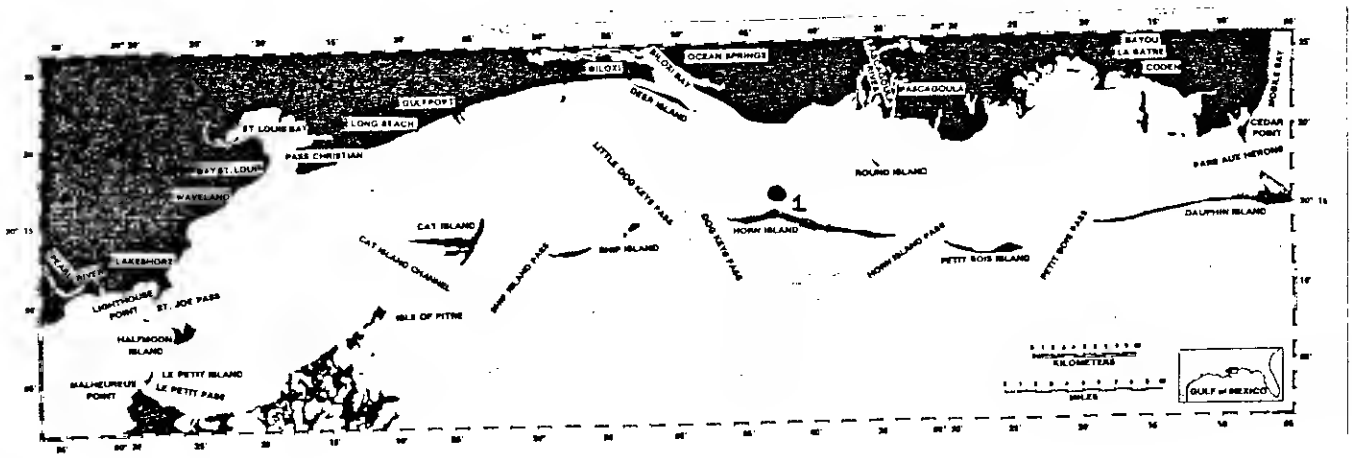
Figure 13: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 638 Sex: F Age: 6+

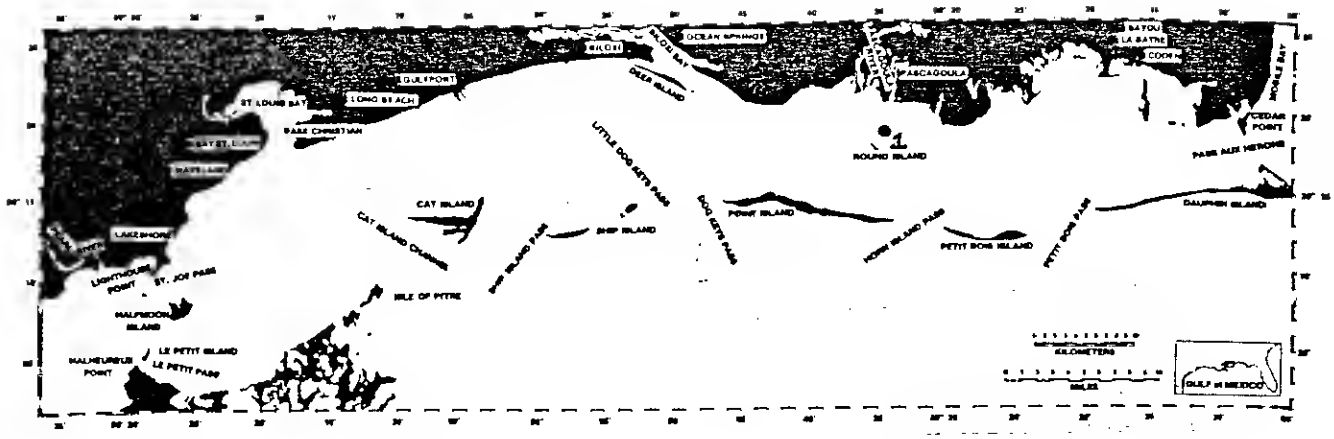


Animal No.: 639 Sex: F Age: 13+

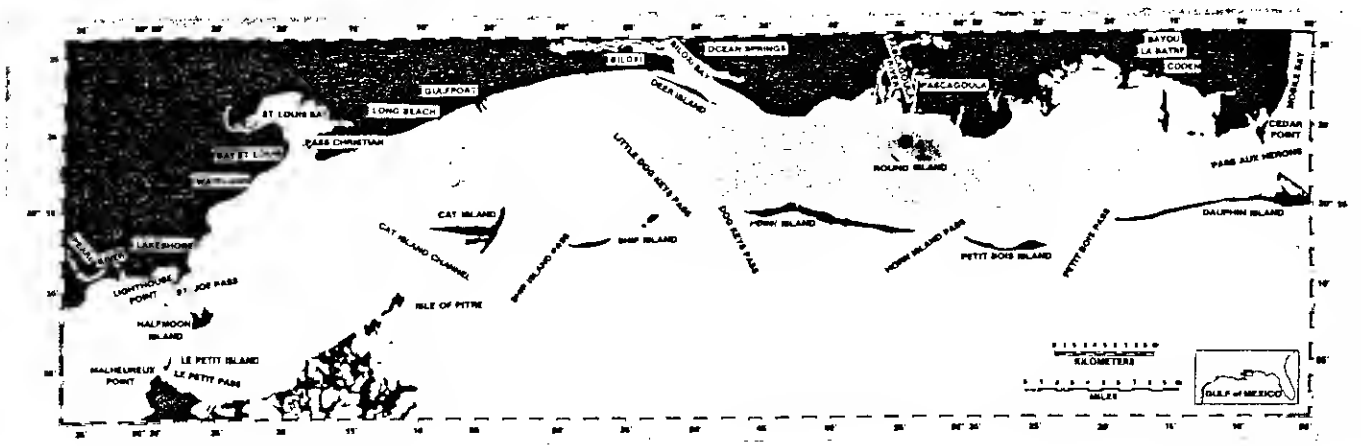


Animal No.: 640 Sex: F Age: 15+

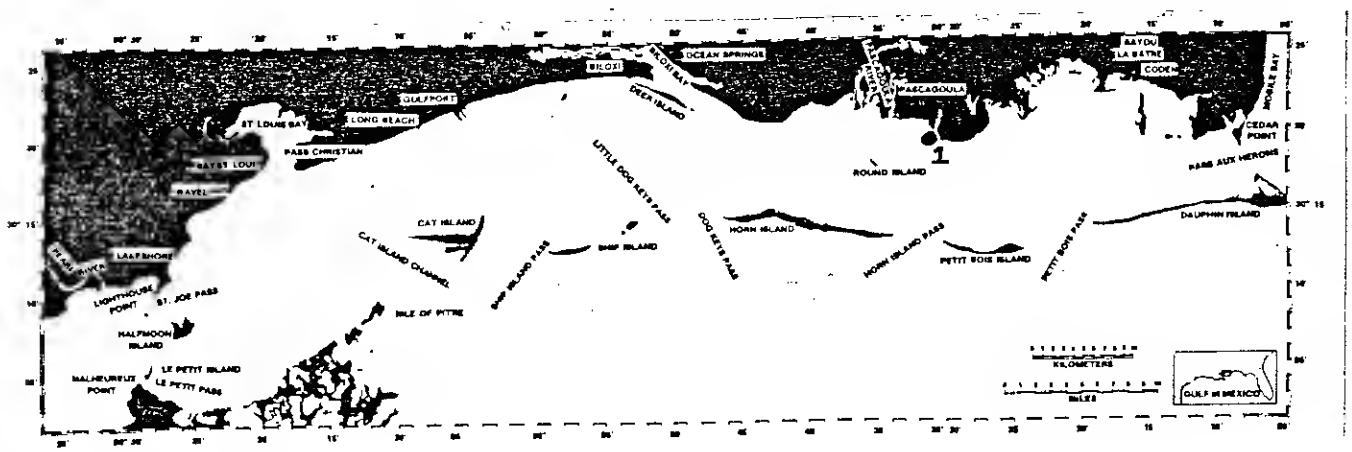
Figure 14: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 642 Sex: M Age: 8

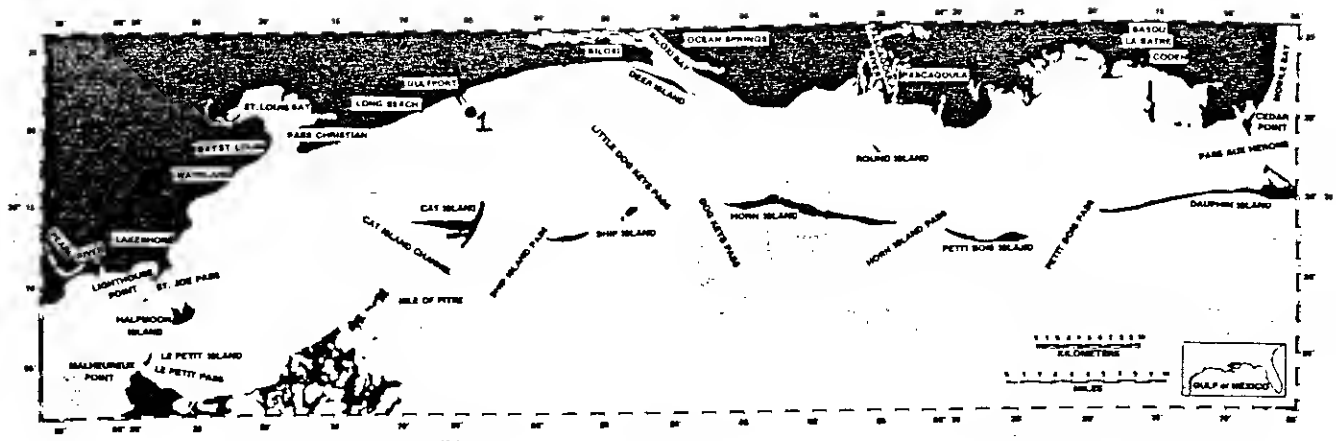


Animal No.: 643 Sex: M Age: 8

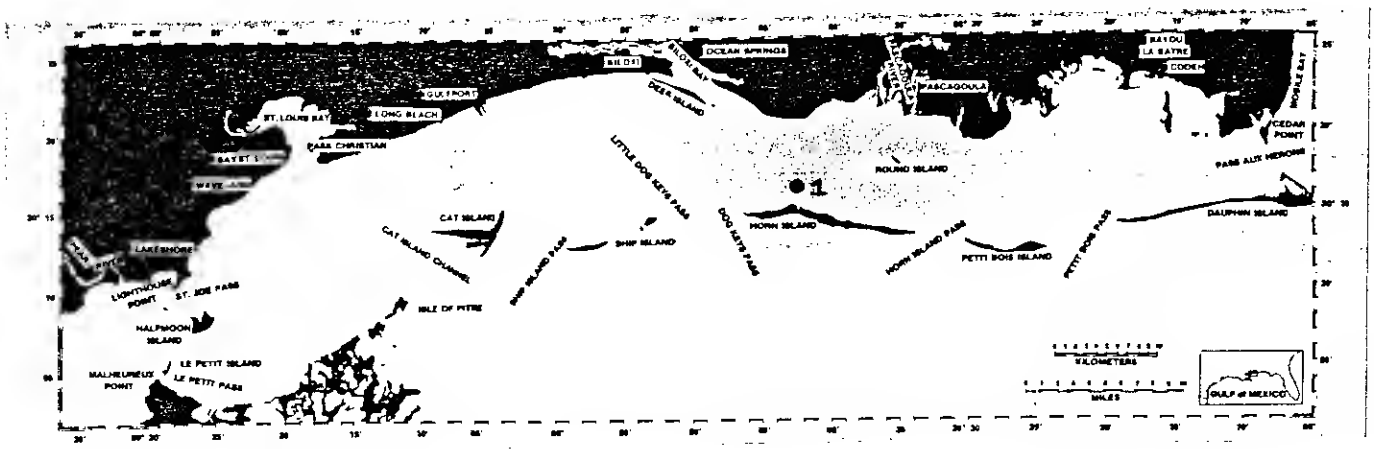


Animal No.: 645 Sex: F Age: 21

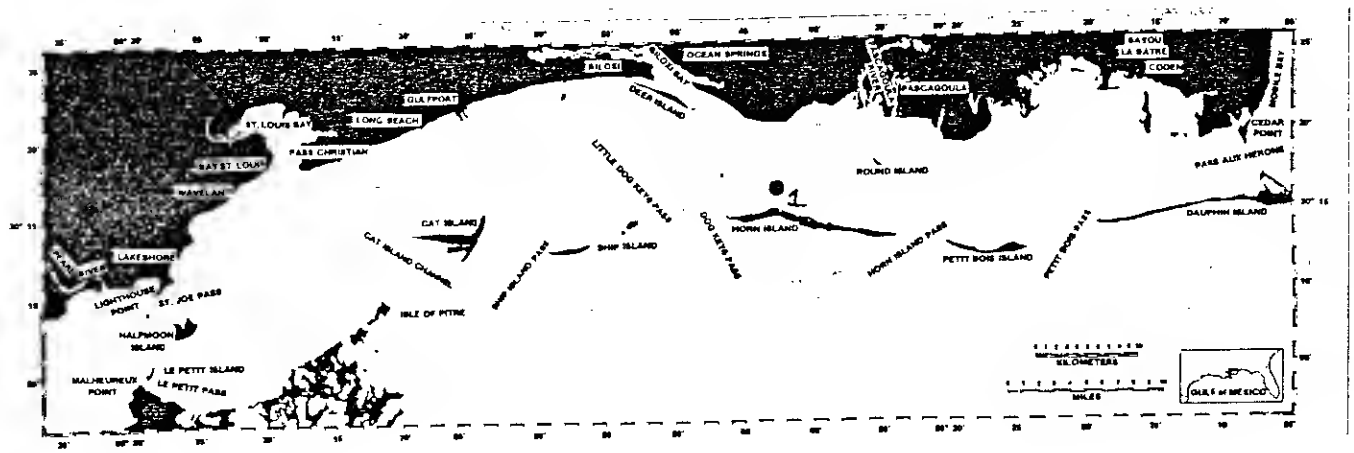
Figure 15: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 647 Sex: F Age: 5

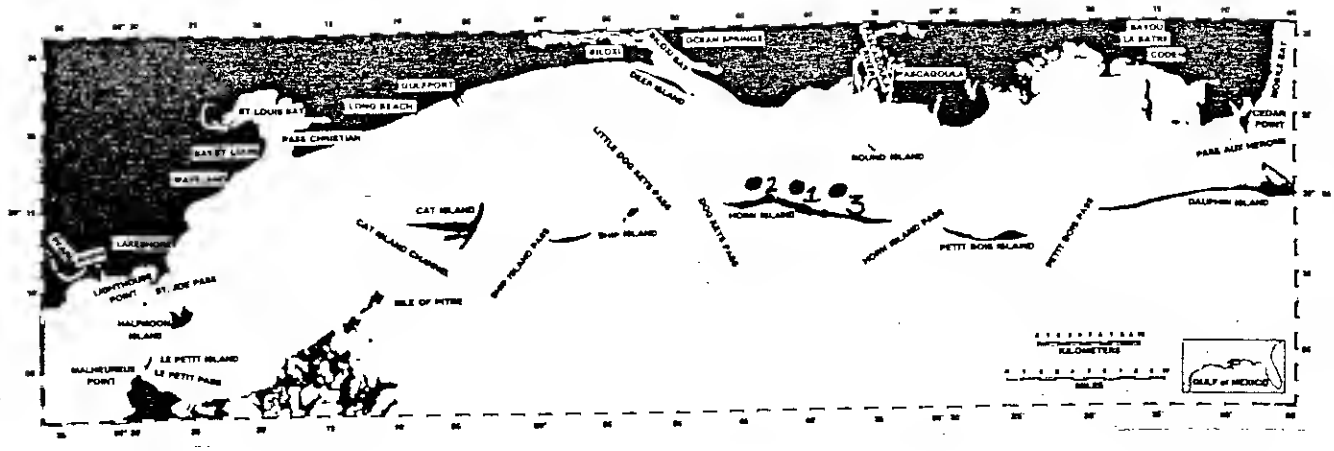


Animal No.: 648 Sex: F Age: 5

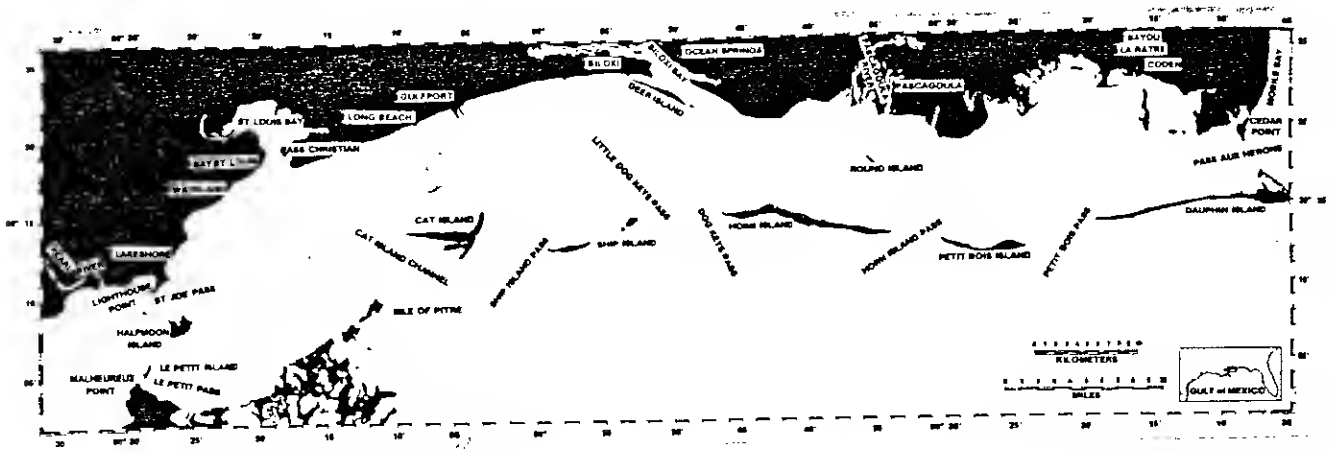


Animal No.: 649 Sex: F Age: 3

Figure 16: Approximate geographical location for individual resighting and frequency of observation for each animal.



Animal No.: 650 Sex: F Age: 5



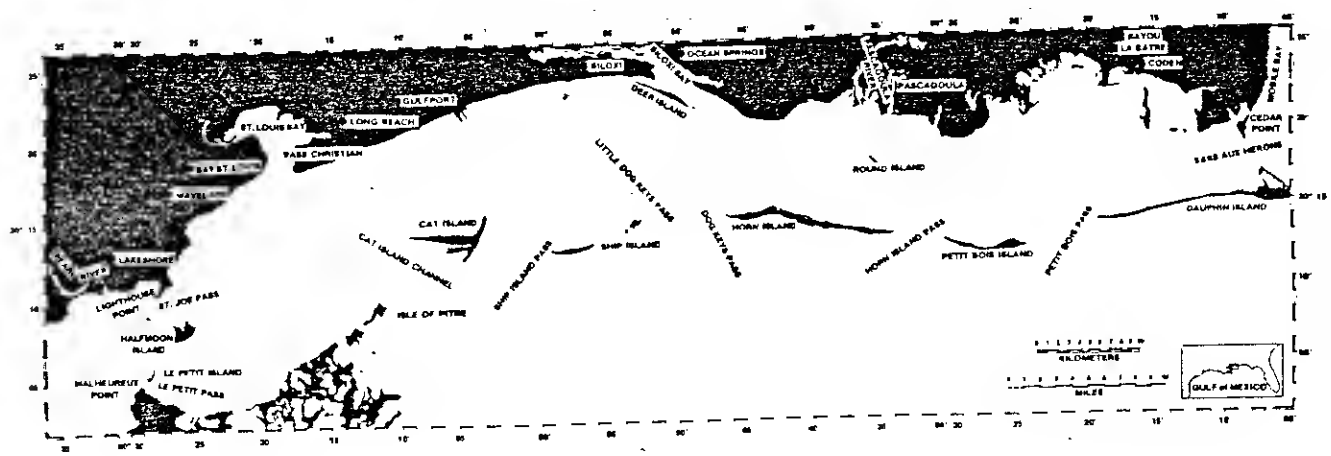


Figure 17: Approximate geographical location for individual resighting and frequency of observation for each animal.

CONCLUSIONS AND RECOMMENDATIONS

1. Freeze marking appears to be an acceptable method to study the population dynamics of Tursiops truncatus in the Mississippi Sound.
2. The capture, handling, freeze marking, and biological sampling procedures did not appear to have any significant or permanent effect on the animals studied.
3. The data base developed for the dolphins sampled from the Mississippi Sound offers an excellent opportunity for comparing data from dolphins sampled from other geographical areas and captive animals.
4. Because of the small sample size and the limited time during which the study was conducted, it is difficult to evaluate and document seasonal changes, especially hormonal variations, and other long term trends.
5. Based on observations made during the collection effort on the abundance of these animals, there appears to be a sizable population of Tursiops in the Mississippi Sound. Furthermore, results from the analyses of various biological samples taken during the study (hematology, serum chemistry, microbiology, endocrinology) seems to suggest a "healthy" population in the Sound.
6. For the successful completion of the study the resighting phase should be funded and carried out. Without these follow-up observations, major portions of the badly needed information on the movement, migration, reproduction, and longevity of these animals will not be available to the scientists or management agencies.

ACKNOWLEDGEMENTS

The successful completion of the project was a result of the participation and assistance of numerous individuals.

Mr. and Mrs. Salvador Giuffria had a major responsibility in providing logistical support for the capture and handling effort. Captains Phil Stevens and Robert Corbin assisted in the capture of the dolphins. Their years of experience in collecting and handling dolphins and their knowledge of Mississippi waters was indispensable. Our exceptional ability in making successful sets is a result of this experience. Commodore Walter Vick was our scout in the air. His efforts in locating herds and directing the ground crews to their vicinity undoubtedly saved the group a lot of time on the water in locating dolphins. The collection, handling, and processing would not have been possible without the fine participation of dolphin trainers and handlers from Marine Life, Marine Animal Productions, and Eight Flags, Inc. Most notable was the assistance of Messrs. M. Wood, D. Klute, K. Sullivan, K. Daniels, J. Bebler, S. Bebler, D. Jones, C. Fairchild, B. Corbin, and Ms. K. Brewer.

Drs. Toom, Middlebrooks, and Odell conducted the biochemical genetics, microbiology, and endocrinology and age analysis, respectively. Even with the budgetary constraints, these gentlemen were able to produce exceptional data from the study. The hematology and serum chemistrys were conducted by the Gulfport Memorial Hospital, Gulfport, Mississippi and the contributions

of Dr. Saccoccia (M.D.), Mr. Hollingsworth, Mr. Cheek, Ms. Slaughter, and Ms. McKinnis are appreciated.

Several scientists from the National Marine Fisheries Service and the Animal Plant Health Inspection Service participated in the project. The efforts and enthusiasm of Mr. Rob Ford, the COTR of the project are especially recognized. Mr. Hoggard and Ms. Manzella went with the group as observers and subsequently participated with Mr. Ford in the resighting phases. Furthermore, the interest of Drs. Kemmerer, Nelson, and Mr. Benigno of the Pascagoula Laboratory of NMFS in the study is highly appreciated. Both Dr. McEnroe (D.V.M.) and Dr. Brown (D.V.M.) of APHIS went with us on the collection trips. Their active participation in the effort and their valuable comments on the collection of samples was commendable.

We would also like to thank Mr. Donald P. Jacobs, President, of Marine Animal Productions for the monetary assistance for the project. His enthusiasm, support, and interest in the study were a tremendous moral booster for the entire group.

Finally, I would like to thank Ms. Barbara Baxter for her exceptional assistance in this project. Her responsibilities included the organization of the data collected on-board and at the laboratory, data compilation and computer analysis, and proofing the manuscript. We certainly appreciate her active participation in the project.

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APPENDIX C: COST ANALYSIS

The cost of collection, marking, and laboratory analysis provided below is based on the capture of 50 dolphins during the thirty day contracted work-period. The total cost includes the services donated by the managements of Marine Life, Inc. and Marine Animal Productions, Inc. toward the study during and after the contracted period. At the time of submission of this report, MAP-Marine Life are continuing to contribute toward the resighting phase of the study that is not being funded by the National Marine Fisheries Service.

CAPTURE AND MARKING:

A.	Boat rental for three adequately equipped boats	$29,000.00
B.	Airplane rental	$ 4,200.00
C.	Boat operating expenses	$ 3,800.00
D.	Price of net	$ 9,200.00
E.	Marking supplies and equipment	$ 3,600.00
D.	Labor: 18 qualified persons including curator, veterinarian, technicians	$58,000.00
	Subtotal	$107,800.00

LABORATORY ANALYSES:

A.	Hematology and serum chemistry	$4,500.00
B.	Microbiology	$3,500.00
C.	Biochemical Genetics	$3,500.00
D.	Endocrinology and Age	$2,000.00
	Subtotal	$13,500.00

DATA ANALYSIS AND REPORT WRITING:

A. Computer time, typing, xeroxing, secretarial.....$5,000.00

Subtotal	$5,000.00
GRAND TOTAL	$126,300.00

SERVICES DONATED BY MANAGEMENTS OF MARINE LIFE-MARINE ANIMAL PRODUCTIONS

A. One laboratory/observation boat (in addition to the three listed above).....$5,000.00

B. Freeze marking and processing of three additional dolphins.....$3,000.00

C. Administrative costs.....$4,500.00

D. Resighting program.....$10,000.00

i. Toll-free telephone lines
ii. Distribution of posters and mail-in cards
iii. Observers
iv. Boat/aircraft time

Subtotal	$22,500.00
GRAND TOTAL	$148,800.00